Joint FAO/WHO Expert Committee on Food Additives (JECFA)

Guidance document for WHO monographers and reviewers evaluating veterinary drug residues in food

Geneva
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List of abbreviations

ADI  acceptable daily intake
ARfD  acute reference dose
BMD  benchmark dose
BMDL  lower 95% confidence limit on the benchmark dose
BMDL_{10}  lower 95% confidence limit on the benchmark dose for a 10% response
BMDs  Benchmark Dose Software (USEPA)
bw  body weight
CAS  Chemical Abstracts Service
CCRVDF  Codex Committee on Residues of Veterinary Drugs in Foods
CD-ROM  compact disc read-only memory
DVD  digital video disc
EDI  estimated daily intake
EHC  Environmental Health Criteria
EMEA  European Medicine Agency
FAO  Food and Agriculture Organization of the United Nations
GEADE  global estimate of acute dietary exposure
GECDE  global estimate of chronic dietary exposure
GLP  good laboratory practice
INN  International Nonproprietary Name
ISO  International Organization for Standardization
IUPAC  International Union of Pure and Applied Chemistry
JECFA  Joint FAO/WHO Expert Committee on Food Additives
JMPR  Joint FAO/WHO Meeting on Pesticide Residues
LC_{50}  median lethal concentration
LD_{50}  median lethal dose
LOAEL  lowest-observed-adverse-effect level
LOEL  lowest-observed-effect level
MIC  minimum inhibitory concentration
MIC_{50}  minimum concentration required to inhibit the growth of 50% of organisms
MIC_{calc}  minimum inhibitory concentration derived from the lower 90% confidence limit for the mean MIC_{50} of the relevant genera for which the drug is active
MOE  margin of exposure
MRL  maximum residue limit
MRL  maximum residue limit
NOAEC  no-observed-adverse-effect concentration
NOAEL  no-observed-adverse-effect level
OCR  optical character recognition
OECD  Organisation for Economic Co-operation and Development
PDF  portable document format
POD  point of departure
ppm  parts per million
QA  quality assurance
SI  Le Système international d’unités (International System of Units)
T_{25}  chronic daily dose that will give 25% of the animals tumours at a specific tissue site, after correction for spontaneous incidence, within the standard lifespan of that species
TMDI  theoretical maximum daily intake
TRS  Technical Report Series
TTC  threshold of toxicological concern
URL  uniform resource locator
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>USB</td>
<td>universal serial bus</td>
</tr>
<tr>
<td>USEPA</td>
<td>United States Environmental Protection Agency</td>
</tr>
<tr>
<td>VICH</td>
<td>International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
</tbody>
</table>
Preface

This guidance document replaces the previous guidance for the hazard evaluation of veterinary drug residues in food by Joint FAO/WHO Expert Committee on Food Additives (JECFA) monographers and reviewers, issued by WHO in 1996. It is intended primarily for WHO Experts (monographers) who prepare monographs for JECFA and for Members (reviewers) who have been assigned to peer review them and propose evaluations. The guidance will also be useful to sponsors who submit dossiers to WHO and other parties interested in understanding the process followed in the evaluation of veterinary drug residues in food by JECFA. Detailed scientific guidance on the interpretation of toxicological and epidemiological data may be found in the monograph Environmental Health Criteria 240 (http://www.who.int/foodsafety/publications/chemical-food/en/).

In this guidance document, reference to JECFA is to JECFA (veterinary drug residues), unless otherwise specified.

With the aim of harmonizing the work of JECFA with that of the Joint FAO/WHO Meeting on Pesticide Residues (JMPR), this guidance document takes into account the document entitled Guidance document for WHO monographers and reviewers, prepared by JMPR in 2015 (http://www.who.int/foodsafety/publications/jmpr_guidance_document_1.pdf?ua=1). The authors of the JMPR guidance document as well as the authors of this guidance document for the evaluation of veterinary drug residues in food are gratefully acknowledged.

It is envisioned that this guidance document will be modified based upon comments received and experience gained in using it. Comments on this guidance document and suggestions for future editions will be gladly accepted by the WHO Joint Secretary, Joint FAO/WHO Expert Committee on Food Additives, World Health Organization, 1211 Geneva 27, Switzerland, at jecfa@who.int.
Chapter 1: Roles and responsibilities

The roles and responsibilities of the JECFA Secretariat and of both monographers (“Experts”) and reviewers (“Members”), from the time they are assigned to their compounds through to the post-meeting finalization of their monographs, are outlined below.

1.1 Selection of compounds on the agenda and issuing the call for data

The compounds on the agenda for the next JECFA meeting on veterinary drug residues in food are selected on the basis of a priority list established by the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF), requests by FAO and WHO and their Member States, and recommendations of earlier meetings of JECFA. The WHO and FAO Joint Secretaries post a call for data on the compounds on the agenda 10–12 months in advance of the meeting on the Internet, utilizing as broad a distribution as possible. The deadline for submission of data is ordinarily 6–7 months before the meeting.

1.2 Identification of monographers and reviewers and assignment of compounds and tasks

The WHO Joint Secretary will contact potential monographers and reviewers within the existing roster of experts about their interest and availability to serve as experts for the next meeting of JECFA on veterinary drug residues in food. Participants are invited as independent experts in their respective areas, and they do not represent any organization or government. Participation is not compensated, although WHO is responsible for return airfare and provides a daily subsistence allowance to cover accommodation, meals and other miscellaneous expenses.

In accordance with WHO rules and procedures for declarations of interest, any potential or perceived interests will be evaluated before any tasks are assigned. In the interest of transparency and to avoid potential conflicts, participants are encouraged to be inclusive in the declaration of interests. It is important to note that the focus should be on a comprehensive declaration of all interests, not just those perceived by the participant as potentially posing conflicts. In accordance with WHO procedures, declarations of interest are not published, but potential conflicts of interest that preclude participation in discussions on particular compounds are noted in the meeting report. The WHO Joint Secretary will take into account whether monographers have been involved with a particular compound, which may be perceived as a conflict or bias. Interests to be considered include the following examples:

- Monographers have worked for or have an interest in the sponsoring company.
- Monographers have performed some of the studies to be evaluated.
- Monographers have recently been closely involved with preparing an evaluation of a compound for a national or supranational body.

The last point is important as, although familiarity with a compound and the supporting data can make preparation of the monograph easier, there might be the perception that the JECFA evaluation is not entirely independent of the previous evaluation.

According to WHO rules and procedures, expert meetings are private in nature, and participation is by invitation only. The data used and discussions held before, during and after the meeting on the subject matter of the meeting are to be held in strict confidence. Discussions held subsequent to the meeting with non-participants should be limited to the public information made available in the monographs and meeting report.

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1 Previously Temporary Advisers.
2 http://www.who.int/about/declaration-of-interests/en/
1.3 Dealing with the data submission

After a compound has been assigned to a monographer and a reviewer, the Secretariat will ensure that the sponsoring company arranges submission of the dossier, which contains the original study reports, relevant papers from the literature and the company overview (summary of the submitted data). As a good practice, the sponsoring company is asked to alert the monographer, the reviewer and the WHO Joint Secretary when the data have been sent. Normally, the data are submitted as searchable PDF files on a suitably indexed CD-ROM, DVD or USB stick. A table of contents using fully descriptive file names needs to be submitted with each electronic submission; for example, a title of “xyz 33564-05” is not going to help the monographer locate a 90-day dog study by Jones et al. (2001). Sponsoring companies should submit editable PDFs whenever possible; when documents are scanned, these should be converted using OCR to editable format, if at all possible. This facilitates the accurate transfer of information to the monograph. Companies should be aware that, owing to the workload of experts reviewing the dossiers, delay of a submission may cause the compound to be removed from the JECFA agenda.

When the data are received, it is important for the monographer to confirm receipt to the sponsor and the WHO Joint Secretary. If the data submission does not arrive within a reasonable period of time, the monographer should contact the sponsor and the WHO Joint Secretary, as it is not unknown for items to go missing in transit. On opening the package, it is recommended that the monographer perform some basic checks on the quality and usability of the documentation:

- For electronic submissions
  - Do the document files open properly?
  - Are a table of contents and an appropriate index provided?
  - Are the files searchable?
  - Are the pages legible, especially older study reports that have been scanned?
  - Are the titles of the files helpful?
- Check the company overview
  - Is it in the JECFA style and in a suitable format (PDF and/or Microsoft Word) to permit the use of text or tables for the monograph?
  - Does it contain a reference list in the JECFA style (see section 2.3.5)?

If the monographer identifies any issues with the data submission where it is believed that the sponsor could provide an improved submission, then the monographer should inform the WHO Joint Secretary, who will contact the sponsor with a detailed request for what is needed. It is in the sponsor’s interest to provide a usable submission. If the monographer cannot read data in a key study report, this might prevent the establishment of a health-based guidance value.

Unpublished confidential studies that are submitted will be safeguarded and will be used only for evaluation purposes by JECFA. Summaries of the confidential studies will be published by FAO and WHO after the meetings in the form of residue and toxicological monographs.

Submitted confidential data can be either returned to submitters at their expense or destroyed after the evaluations have been completed. Key material can be stored by WHO for up to five years and then destroyed.

1.4 Handling contacts with the sponsor

To ensure transparency, it is important that all contacts between the monographer and the sponsor are documented and copied to the WHO Joint Secretary. With respect to contact with the sponsor:

- It is preferable to use email rather than telephone. Emails need to be cc’ed to the WHO Joint Secretary.
- If the sponsor telephones to discuss an issue, the monographer should consider whether the discussions can be performed by email. If the monographer chooses to proceed with the call, the monographer should notify the WHO Joint Secretary about the contact, with a brief outline of the details. If a teleconference is requested or considered useful, the monographer should involve the WHO Joint Secretary, who will set up the call.
- The sponsor should assist the monographer by providing information required to perform a thorough and independent evaluation. The monographer may send questions to the sponsor,
copied to the WHO Joint Secretary, well in advance of the meeting, as well as, on occasion, during the meeting itself.

- The sponsor must not contact the monographer with repeated requests for progress updates or for information that is not appropriate to be shared, such as the health-based guidance value; if this occurs, the monographer should notify the WHO Joint Secretary.

1.5 Performing literature searches

In addition to the unpublished study reports and other material submitted by the sponsor, a search of the public literature is required to ensure that all available information is being considered in the evaluation. The monographer is requested to perform a detailed search of the public literature. The literature search should be documented in detail, listing the exact search terms used, the databases that were searched, the number of references retrieved and the number of relevant references selected, as well as the criteria (both inclusion and exclusion) for the selection of relevant references. The WHO JECFA Secretariat can assist in developing search strategies and in retrieving the full text of relevant publications.

1.6 Evaluating the data

The basic principles on how to evaluate toxicological and epidemiological data are outlined in Environmental Health Criteria 240 (IPCS, 2009). A JECFA monographer will already be an experienced assessor of toxicological and epidemiological data and will have his or her own ways of working through the toxicological and epidemiological database on a compound, including submitted data and publicly available information. Based on a detailed search of the JECFA and JMPR databases, the monographer should identify previous evaluations of the compound or of its metabolites by both JECFA and JMPR, even if such an assessment was based on a different denomination or chemical name. This is particularly important if the chemical has been used as both a veterinary drug and a pesticide.

The JECFA process should not require any significant changes to the monographer’s and reviewer’s usual way of working through the data, provided that each study is described and the relevance (including any potential bias or problems with study design or reporting of results) is documented in a clear and transparent manner. One important difference for monographers from a regulatory agency background is that “stop the clock” and demand for new studies are not foreseen; even when major deficiencies are identified, a monograph summarizing available data and clearly outlining the deficiencies may need to be prepared. When the monograph is being prepared, all data are evaluated in a thorough and independent manner, taking into account specific guidance prepared for JECFA monographers on the interpretation of toxicological and epidemiological data (i.e. EHC 240 [IPCS, 2009] and subsequently published guidance).

The depth of investigation will clearly vary with the study type, the results and the impact on the overall conclusion. For example, it can be valuable to go down to individual animal-level data for a dog study with a small group size and a marginal response, but this is not normally required for a rodent study with a larger group size and clear effects (e.g. 8/10 animals with grade 3 versus 3/10 controls with grade 1). In general, the monographer should always check at least the results in the main study report, and not just the sponsor’s or study report authors’ summary. If the study report authors have discounted particular findings as not being treatment related or adverse, the monographer should pay particular attention to these to see if he or she agrees with the study report authors’ conclusions. If the monographer disagrees with the conclusions of the study report authors, this should be highlighted in the monograph.

In presenting findings where descriptive terms are used, it is important to use the precise terms as given in the study report (e.g. in the histopathology tables or descriptions of anomalies in developmental toxicity studies). If for any reason a revised term is used, there should be some

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4 The JECFA Secretariat is currently investigating the applicability of systematic review methodology to the work of JECFA, with the ultimate aim of developing a workable approach that is manageable and follows the basic principles on transparency, minimizing risk of bias and reproducibility.

JMPR: http://apps.who.int/pesticide-residues-jmpr-database
commentary about this, as it can produce confusion for someone comparing reviews with the study report. If the term is an unfamiliar or unusual one that is not clarified in the study report, then there is the option to ask the sponsor to clarify and/or provide pictures. Standard texts and websites are available that provide descriptions of pathological and developmental toxicity terminology (e.g. http://www.devtox.org; http://www.goreni.org; see also the guidance below under specific systems and effects).

JECFA has developed specific criteria for the interpretation of certain effects observed in toxicity studies (e.g. avermectins in the CF-1 mouse, pharmacological rather than toxicological end-points, antimicrobial effects and injection sites) and has adopted guidance on the interpretation of other effects from JMPR (e.g. guidance on the interpretation of hepatocellular hypertrophy) (see Chapter 4). Where JECFA has its own criteria for the interpretation of end-points (i.e. EHC 240 [IPCS, 2009] and subsequently published guidance), these should always be used in the preparation of monographs in preference to those from national or other supranational bodies. Where JECFA does not have its own criteria, then general guidance on the evaluation and interpretation of toxicological data available in the WHO EHC monographs and elsewhere may be used. It is expected that standard approaches will be applied (e.g. statistical significance, clear dose–response relationship, change outside the normal biological range). If a conclusion in a monograph is based on a non-standard approach (e.g. the use of a specific cut-off), then the basis for this approach should be provided (or a publicly available supporting guidance document should be cited).

It is important to remember that JECFA considers the establishment of both chronic (acceptable daily intake [ADI]) and acute (acute reference dose [ARfD]) guidance values, as appropriate (for more information on ARfDs in the context of veterinary drug residues, see section 3.5). Where appropriate, acute dietary exposure estimates should cover a time period of food consumption over a single meal or 24 hours and are intended to be used for comparison with ARfD values in a risk assessment process. In respect of the ARID, it is important to consider data at the earliest measurement period in a study and the results of short-term or acute studies if appropriate measurements have been performed. It is also important to confirm that the level of investigation in targeted studies, such as the acute neurotoxicity study, is adequate to address the end-points seen in longer-term studies. For example, if the critical finding in a 90-day rat study is haemolysis, but blood samples were not taken in the acute neurotoxicity study, then the acute neurotoxicity study might not be an appropriate basis for establishing an ARID.

1.7 Preparing the draft monograph before the meeting

The monographer produces a first draft of the monograph, based on the submitted dossier as well as a critical review of the published literature. Each monograph includes a main body of text as well as an Explanation section, a Comments section and an Evaluation section; these three sections will be used as the basis for the meeting report item for the compound (see Chapter 3). Detailed guidance on preparation of the monograph is provided in Chapter 2. Examples of recent toxicological monographs can be accessed through the WHO JECFA searchable database: http://apps.who.int/food-additives-contaminants-jecfa-database/search.aspx?fc=35.

In cases where new or additional data are provided to complete an evaluation or to re-evaluate a compound previously considered by JECFA or to establish an ARID that was not previously considered, an addendum to the original monograph should be prepared with the summaries of the new studies. A short monograph addendum is usually prepared even when the number of studies available is low. However, in specific instances, this may be considered unnecessary – for example, where the additional information is restricted to a narrow aspect of the evaluation and does not have any impact on the previously established health-based guidance values. In such cases, only the meeting report item is prepared.

The first draft of the monograph is distributed first to the reviewer. The reviewer should receive the first draft of the monograph at least three months before the meeting. The monographer and reviewer are encouraged to work together and discuss critical aspects or studies throughout the preparation of the monograph. It is the responsibility of the reviewer to cross-check critical studies, suggest amendments in both text and tables, and finalize the Comments and Evaluation sections.

The reviewer returns the monograph to the monographer, who incorporates agreed changes into the document and then sends the revised draft monograph to the WHO Joint Secretary. During the preparatory phase, usually 4–6 weeks before the meeting, the WHO Joint Secretary organizes
teleconferences for each compound, involving at least the monographer, the reviewer, other JECFA experts, including FAO experts assigned to the same compound, and the WHO Joint Secretary. The purpose of these teleconferences is to clarify issues, coordinate the work between the WHO and FAO experts and identify additional information or clarifications required from the sponsor. A list of any outstanding questions is established for each compound and sent to each corresponding sponsor. The monographer is also responsible for making any additional revisions suggested by teleconference participants.

After the final revisions have been made to the draft monograph, the monographer sends a copy of the monograph, without the Evaluation section (containing the proposed health-based guidance values), to the sponsor for an accuracy check of study descriptions, to be completed within two weeks. Any comments received have to be considered by the monographer in discussion with the reviewer.

The final draft monograph is then submitted to the WHO Joint Secretary, who is responsible for sending the draft monograph to all meeting participants at least 10 days prior to the meeting.

1.8 Preparing the report item and finalizing the monograph at the meeting

The physical meeting is organized jointly by FAO and WHO and generally alternates between Rome and Geneva. During the meeting, the monographers lead the discussions on their particular compounds and prepare the meeting report item for each compound under their responsibility. The report item is prepared from the Explanation, Comments and Evaluation sections of the monograph (see Chapter 3) and is modified during the meeting to incorporate the results of the meeting discussions. In parallel, during the meeting, the monographer updates the draft monograph to ensure that the final version is consistent with the meeting report item, to reflect decisions taken during the meeting (e.g., decisions on the NOAELs) and to include any extra details found to be useful in supporting the conclusions of the Committee. Note that when both toxicological and residue evaluations performed for any veterinary drug at the same meeting, there will be a common Explanation section in the final meeting report item, which includes both WHO and FAO evaluations. Hence, WHO and FAO monographers should work together, both before and during the meeting, to ensure that the final version of the Explanation section reflects the necessary information from both groups, in a consistent manner.

It is the JECFA Members who have the final responsibility for adopting the report. However, during the meeting, conclusions and decisions are reached by consensus from all participants. Therefore, all monographers and reviewers should contribute to discussions on all the compounds and general considerations. This is particularly the case if the monographers have expertise in a specific area (e.g. histopathology, genotoxicity, developmental toxicity, microbiology), such that they can bring additional insights and views to the discussions. It is also important that monographers ask questions when they are unclear about the basis for a decision or if the text relating to a topic is not well presented. However, monographers need to be aware that their report items must be completed prior to the conclusion of the meeting and so must carefully balance the requirement for the timely preparation of drafts of their report items for discussion at the meeting and contributing to discussions on other compounds.

During the meeting, the rapporteur is responsible for ensuring that all necessary revisions resulting from discussions have been made to each draft report item before the item is again discussed by the Committee. The editor is responsible for technical and language editing of each draft report item once the Chair is satisfied that it is in near-final form. The monographer is responsible for responding to any queries raised by the editor during the editing process. After the meeting report item has been edited, subsequent changes, as suggested by meeting participants during discussions, will be tracked onscreen by the editor, until the Chair is satisfied that the meeting report item is in final draft form. At that time, the editor passes the report item on to the FAO rapporteur for FAO review and incorporates any changes suggested by FAO. Additional editing may be performed by the editor after the meeting has concluded (see below).

On the last day of the meeting, all meeting participants (FAO and WHO) review the final version of the meeting report in a plenary session and suggest any necessary revisions, which are usually made onscreen by the editor or by the WHO Joint Secretary, and the JECFA Members formally adopt the report before the meeting is adjourned. The monographer needs to provide an electronic version of the final draft of the monograph to the editor and the WHO Joint Secretary before the end of the final meeting day. There is no need for the monographer to update the Explanation, Comments and
Evaluation sections of the monograph during the final session, as the editor will insert the final versions of those sections from the meeting report into the monograph during the editing process.

In the weeks following the meeting, a summary report is published and posted on the FAO and WHO websites. It includes the main conclusions, the health-based guidance values (i.e. ADIs and ARfDs) or other safety recommendations and the proposed maximum residue limits (MRLs) for all veterinary drug residues evaluated at the meeting.

In the months following the meeting, the editor edits the toxicological monographs. The monographers are responsible for answering any queries raised during the editing process in a timely fashion (generally within 1–2 months after receiving the monograph back from the editor). The meeting report is not published until the monographs have been edited, so that any errors in the meeting report found during the editing process can be corrected before its publication.


The meeting report is intended for non-experts (both policy-makers and risk managers) and contains the description, concise evaluation and interpretation of the key data relevant for the overall assessment of each substance reviewed by JECFA in terms of its toxicological, microbiological, epidemiological, chemical and analytical aspects, as well as information on the dietary exposure assessment. Reports reflect the agreed view of the Committee as a whole and describe the basis for its conclusions. Any Members who do not agree with the conclusions can express a minority opinion, which should be noted and described in detail in the meeting report, in accordance with WHO rules and procedures for expert committees.

The toxicological monographs are intended for experts and contain detailed descriptions of the full database on biochemical, toxicological, microbiological and epidemiological data considered in the evaluation, as well as the dietary exposure assessment, in sufficient detail to enable the basis of the conclusions reached by the Committee to be independently verified. The Comments and Evaluation sections of the monographs are in principle identical to the report item (with the inclusion of the Explanation section). In exceptional cases, these sections could contain more detail than in the report.
Chapter 2: Preparing the monograph

2.1 Introduction

The monograph contains the detailed study descriptions and numerical data used to underpin the meeting report item, referred to below as the report item (see Chapter 3). The monograph must therefore contain all the elements identified in the report item, together with sufficient additional details to permit an independent evaluation of the conclusions made. A table of contents or template for the monograph (for the JECFA current year) will be provided to monographers when they are allocated a compound. An example is included in Annex 1. The layout and sequence of the template should generally be followed. The template for the current year should always be used, as modifications may have been introduced following the previous meeting.

2.2 General aspects

General aspects to be considered while preparing the monograph are outlined below.

2.2.1 Formatting

- The monograph (or monograph addendum) should be prepared using Microsoft Word or a compatible word processing package. Details of the formatting requirements (e.g. font size, line spacing, line numbering, margins) should be obtained from the monograph template (see Annex 1).
- Details of the formatting requirements for preparing tables are provided in section 2.2.5.

2.2.2 Units of measurement

- Le Système international d’unités (SI units) should be used throughout. This includes the use of milligrams per kilogram of feed (mg/kg feed) instead of parts per million (ppm) for dietary exposure levels and the use of becquerels (Bq) instead of curies (Ci) for radioactivity. One exception is millimetres of mercury (mmHg) for pressure (the equivalent in kilopascals [kPa] should be given in parentheses).
- When expressing dietary exposure levels in milligrams per kilogram, the word “feed” should always be included (i.e. mg/kg feed), to avoid confusion with the actual dose to the animals (in mg/kg bw, where bw is body weight).
- There are no hyphens between numbers and units, but there is a space. For example, 0.5 kg rat (not 0.5-kg rat or 0.5kg rat) is used.
- There should be no words between units and the solidus (/). For example, 3 µg derquantel/kg bw is not correct. Instead, the sentence should be rewritten as, for example, “a derquantel dose of 3 µg/kg bw”. It is recognized that there may need to be occasional exceptions to this rule in order to avoid extremely awkward wording.
- Only one solidus should be used. For example, 3 mg/kg bw per day, not 3 mg/kg bw/day, is used.
- Figures with more than four digits use a space (not a comma) to separate groups of three digits on either side of the decimal point (e.g. 12 050; 0.004 56). Note that the WHO rule is that in tables, figures with more than three digits use a space to separate groups of three digits on either side of the decimal point. The WHO rule is to be followed, even though the guide for the use of SI units (Thompson & Taylor, 2008) states that the practice of inserting spaces in numbers having only four digits on either side of the decimal marker is not usually followed except when uniformity in a table is desired.


2.2.3 Presentation of doses

- Parentheses, rather than commas, are used when presenting dose conversions: $X$ and $Y$ mg/kg feed (equal [or equivalent] to $x$ and $y$ mg/kg bw per day for males and $a$ and $b$ mg/kg bw per day for females, respectively).
- “Equal to” is used when the conversions have been calculated using feed or drinking-water consumption and body weight data generated for the animals that have been dosed in a particular study, and “equivalent to” is used when dose conversion factors (i.e. default values) have been used to calculate the doses.
- Where accurate doses cannot be calculated on the basis of measured body weights and feed or drinking-water consumption, approximate doses can be estimated using the dose conversion factors shown in Table 1, adapted from Environmental Health Criteria monograph 240 (EHC 240; IPCS, 2009).
- When doses are converted from ppm, mg/kg feed, mg/L, mg/animal per day or percentage of the substance in the diet (often given when the lowest dose is 1000 mg/kg feed or more) to mg/kg bw per day, up to two additional significant figures can be used for the converted dose, where necessary, to avoid introducing additional uncertainty in the calculation of the final rounded ADI or ARfD.
- As long as the dose conversions have been presented at the beginning of a study description, the original doses (e.g. in mg/kg feed or percentage in the diet, but not in ppm, which must be changed to mg/kg feed or mg/L drinking-water) can be used throughout the study description until the no-observed-adverse-effect level (NOAEL) is identified at the end of the study description.
- Equivalent doses should be corrected for the purity of the compound, but only when this is less than 90%.
- Doses should be corrected for non-continuous dosing (e.g. 5 days/week dosing).

2.2.4 Presentation of point of departure (POD)

- Health-based guidance values (i.e. an ADI or an ARfD) are most often established using a point of departure (POD) from a toxicity study in experimental animals. The most frequently used POD is the NOAEL. However, if the data are adequate to permit dose–response modelling, a lower 95% confidence limit on the benchmark dose for an $x\%$ response (BMDL$_x$) or similar POD can (and should) be used. In such cases, the basis for the derivation of the POD should be provided (for details, see EHC 240; IPCS, 2009).
- Past tense should be used when presenting the POD: The NOAEL/BMDL$_x$ was 10 mg/kg bw per day.
- The POD used in risk assessment by the Committee should be that identified by the monographer/Committee. When this differs from the POD identified by the study authors, the latter should also be reported, with an explanation for the difference.
- When doses have been derived from a dietary concentration using the feed or drinking-water consumption and body weight data from the study, the POD should be expressed as “equal” to $x$ mg/kg bw per day. If predefined dose conversion factors (see Table 1 above) have been used, the POD should be expressed as “equivalent” to $x$ mg/kg bw per day.
- The POD, as either “equal to” or “equivalent to” doses, can be (but does not have to be) provided for both males and females in the main text, but only the lower value of the two (usually the value for males) is used in the Comments section. An exception to this rule is where the effect is sex specific, in which case the appropriate POD for the sex in which the effect is observed is provided.
- The general statement will read as follows: When the POD is the NOAEL: The NOAEL was $x$ mg/kg feed (equal to $y$ mg/kg bw per day), based on [effects] observed at $z$ mg/kg feed (equal to $a$ mg/kg bw per day) [where $z$ mg/kg feed is, of course, the lowest-observed-adverse-effect level (LOAEL), but this does not need to be stated explicitly in the text]. When the POD is the
Table 1: Approximate relationship of mg/kg (ppm) in the diet or mg/L (ppm) in drinking-water to mg/kg bw per day

<table>
<thead>
<tr>
<th>Species</th>
<th>Body weight (kg)</th>
<th>Feed consumption (g/day)</th>
<th>Type of diet</th>
<th>1 mg/kg in diet is equivalent to x mg/kg bw per day</th>
<th>Water consumption (L/day)</th>
<th>1 mg/L in water is equivalent to x mg/kg bw per day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>0.02</td>
<td>3</td>
<td></td>
<td>0.150</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Dry laboratory chow diets</td>
<td>0.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.006&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.20&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Rat (young)</td>
<td>0.10</td>
<td>10</td>
<td></td>
<td>0.100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat (multigeneration studies)</td>
<td>0.10–0.40&lt;sup&gt;d&lt;/sup&gt;</td>
<td>10–20&lt;sup&gt;d&lt;/sup&gt;</td>
<td>(average: 0.25)</td>
<td>0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat (old)</td>
<td>0.40</td>
<td>20</td>
<td></td>
<td>0.050</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hamster</td>
<td>0.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td>0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.21&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chick</td>
<td>0.40</td>
<td>50</td>
<td></td>
<td>0.125</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guinea-pig</td>
<td>0.75</td>
<td>30</td>
<td></td>
<td>0.040</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.84&lt;sup&gt;b&lt;/sup&gt;</td>
<td>34&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td>0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.24&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Rabbit</td>
<td>2.0</td>
<td>60</td>
<td></td>
<td>0.030</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>186&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td>0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.41&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.11&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dog</td>
<td>10.0</td>
<td>250</td>
<td></td>
<td>0.025</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>300&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td>0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.61&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.05&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cat</td>
<td>2</td>
<td>100</td>
<td>Moist, semi-solid diets</td>
<td>0.050</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>168&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td>0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.10&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Monkey (e.g. rhesus, cynomolgus)</td>
<td>5</td>
<td>250</td>
<td></td>
<td>0.050</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhesus monkey</td>
<td>8.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>320&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td>0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.53&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.07&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dog</td>
<td>10</td>
<td>750</td>
<td></td>
<td>0.075</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td>60</td>
<td>1 500</td>
<td></td>
<td>0.025</td>
<td></td>
<td>0.033</td>
</tr>
<tr>
<td>Pig or sheep</td>
<td>60</td>
<td>2 400</td>
<td>Relatively dry grain forage mixtures</td>
<td>0.040</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pig</td>
<td>80&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2 250&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td>0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.07&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cow (maintenance)</td>
<td>500</td>
<td>7 500</td>
<td></td>
<td>0.015</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cow (fattening)</td>
<td>500</td>
<td>15 000</td>
<td></td>
<td>0.030</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Horse</td>
<td>500</td>
<td>10 000</td>
<td></td>
<td>0.020</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

bw: body weight; ppm: parts per million

<sup>a</sup> Liquids omitted.
<sup>b</sup> From Health Canada (1994). Note that the type of diet has not been specified in this reference.
<sup>c</sup> EFSA (2012) uses conversion factors of 0.18, 0.15 and 0.09 for mice for subacute, subchronic and chronic studies. The first two types of studies are assumed to start with mice 5–7 weeks of age.
<sup>d</sup> Body weight and feed consumption values vary over the stages and generations of the studies. The average values are used in calculating the dose conversion factor.
<sup>e</sup> EFSA (2012) uses conversion factors of 0.12, 0.09 and 0.05 for rats for subacute, subchronic and chronic studies. The first two types of studies are assumed to start with rats 5–7 weeks of age.
**BMDL, or similar**: The BMDL [or similar] was $y$ mg/kg bw per day, based on [the effects that serve as the basis of the benchmark response $x$].

- When no effects are observed up to the highest dose tested, it is not possible to determine a BMD. In such cases, the highest dose tested is the NOAEL, which serves as the POD for this study. Consistent language should be used when expressing the NOAEL in such circumstances: The NOAEL was $x$ mg/kg bw per day, the highest dose tested. OR The NOAEL was $x$ mg/kg feed (equal to $y$ mg/kg bw per day), the highest concentration tested.

- When effects are observed at all doses, it is not possible to identify a NOAEL. In such cases, it might be possible to determine a BMD or similar POD. Otherwise, the POD for the study is the LOAEL. Consistent language should be used in such circumstances: No NOAEL could be identified, as effects were observed at all doses. The LOAEL was $x$ mg/kg bw per day, the lowest dose tested. OR The LOAEL was $x$ mg/kg feed (equal to $y$ mg/kg bw per day), the lowest concentration tested.

- If an effect is considered not relevant for determining the POD for a study, a statement should always be made on the reason for this – for example, the effect was considered not to be toxicologically relevant or the effect was considered not to be substance related (with an explanation as to why, if possible), to make the basis for POD determination clear to the reader.

- Determination of overall NOAELs/BMDs (see section 4.5 for definition) is generally reserved for the *Comments* section.

### 2.2.5 Tables

- It is often preferable to present numerical information in the form of a table rather than in the text.

- It is the choice of the monographer as to whether data are presented in tables or text, as long as it is possible for readers of the monograph to perform an independent evaluation of the data and reach their own conclusions.

- Pasting tables from PDF documents into the monograph is not recommended and should be done only if there is no realistic alternative (in which case the editor will need to re-enter the tables in Microsoft Word format in order to edit them according to WHO style). If the sponsoring company has not provided tables in Word format, it should be requested to do so by the monographer. Tabs should not be used to create the table columns.

- All tables must be cited in the text, in consecutive numerical order from 1 to $x$.

- Tables should be placed in the text immediately following the paragraph in which they are first cited, or as near to this as is practical. Repeating header rows can be used where the table extends over more than one page.

- The contents of a table should be restricted to the data relevant to decision-making. If a 200-row table contains 16 rows of data that show no changes with dosing, it is difficult to identify the data that are important.

- There should be no blank cells in the table (unless the cells are in a heading row). If a cell does not contain text or figures, then a 0, dash, NA (for not applicable or not available) or ND (for not determined or no data), or something along these lines, is needed, depending on the table, with a clear definition of the terms used included below the table, if necessary.

- Data in tables should be quoted to an appropriate number of significant figures (e.g. quoting organ weights relative to body weight to six significant figures is not appropriate, as it implies spurious accuracy – six significant figures implies that a change of 0.0001% could be determined with confidence and is biologically significant). The appropriate number of significant figures to be used may vary with the situation but should be sufficient to show differences in outcome while being proportionate to the variance (or standard deviation).

- In some instances, it may be useful to include the standard deviation (or ranges) in addition to mean values.
• An indication of statistical significance should be included wherever appropriate. Boldface type to indicate a statistically significant treatment-related effect can be used, but must be explained in a footnote. Alternatively, superscripts such as * and ** may be used to indicate statistical significance, with definitions included below the table (see next bullet point).

• A listing of all abbreviations used in the table is included below the table, in alphabetical order (e.g. BUN: blood urea nitrogen; Hb: haemoglobin), immediately followed, on the same line, by a description of any P-values used (e.g. *: $P < 0.05$; **: $P < 0.01$), together with a description of the statistical test used in parentheses (e.g. Fisher exact test).

• Table notes (given with lowercase superscripted letters: a, b, c…) appear immediately below the listing of abbreviations. Table notes should be inserted manually, not using the Word footnote function. Within the table itself, lettered table notes are to appear sequentially in alphabetical order, reading across and then down the table (i.e. row by row).

• The table source (Source: Smith & Jones (1999)) is given below the abbreviations and any table notes (superscript a, b, c…). Note that permissions to reprint (to be requested by the WHO Joint Secretary) are required for any tables (or figures) that are taken directly from published sources. Given this requirement, it is preferable to avoid direct copying of illustrative material (tables and figures) from published sources wherever possible.

• Additional miscellaneous points relating to table formats follow:
  o Columns of figures are aligned to the decimal point, where possible. Columns of text are aligned at the left-hand side. The alignment of columns of figures and text combined should be decided on a case-by-case basis.
  o Column headings may be set left or centred over the columns as appropriate (usually centred when the columns contain figures and aligned at the left-hand side when the columns contain text). The first column heading is normally aligned at the left-hand side. Column headings should increase in number from the top to the bottom (e.g. one column heading over three subheadings, each of which is itself over two sub-subheadings). All column headings are aligned at the bottom of the header rows.
  o Column headings are in boldface type.
  o Figures with more than three digits on either side of the decimal point should have a space inserted after each group of three digits (e.g. 3 500; 0.002 3). This rule applies to tables only (in the text, figures with more than four digits have a space after each group of three digits). As noted above, this is not an SI requirement, but a WHO one.
  o Each table entry should occupy its own row to ensure that alignment remains correct when the table is edited.
  o It is preferable to have only one or two row heading levels, in which case the first row heading is flush left and the subheading is indented below it.
  o Where several different row heading levels are needed in the first column, the general order of heading is (1) bold, (2) roman and (3) indented roman (where three levels are needed), (1) bold, (2) italics, (3) roman and (4) indented roman (where four levels are needed) and (1) bold, (2) italics, (3) roman, (4) indented roman and (5) roman following a dash (where five levels are needed). The bold heading row may be shaded for emphasis.

• Some examples of table formats are provided in Annex 2. Additional examples may be found in published JECFA monographs (http://www.who.int/foodsafety/publications/jecfa/en/).

2.2.6 Historical control data

• Historical control data should be reported if considered useful and appropriate for interpreting study findings.

• Historical control data are often presented for tumours and developmental effects, but can be used in an attempt to determine whether the results for any end-point in test animals fall within the normal biological range.

• Historical control data are most useful when they are from the same strain of animal, come from the same laboratory and are reasonably contemporary to the study with which they are being compared (ideally from two years before the start of the study to two years after the end of the in-life phase). If they do not match these criteria, this should be identified in the text.
• If possible, the historical control data should have been submitted such that the results in each study in the database can be seen separately. As an absolute minimum, the number of studies must be given together with the mean and range (just the upper range is not acceptable, as this could be skewed by one atypical study).

• The monographer should seek confirmation from the sponsor that there were no changes in interpretative or investigative techniques between the historical control studies and the one on the test compound.

• If the submitted historical control data do not match these criteria, the sponsor should be asked to clearly identify the differences.

2.2.7 In-text references

• References are cited by one (Brown, 1999), two (Brown & Jones, 1999) or three authors (Brown, Smith & Jones, 1999), or first author plus et al. for four or more authors (Brown et al., 2000). Note the use of an ampersand instead of the word “and” and the use of a comma before the year.

• If the same author(s) published more than one reference in the same year, a, b, etc. should be used to differentiate between the references (Jones & Brown, 1999a,b; Smith, 2000c). This rule also applies to et al. references, even if the other authors are not the same in each reference (Brown et al., 1999a,b).

• In the rare case where different authors with the same surname have published a paper in the same year, initials are used to differentiate between the references (Y. Li et al., 2000; R. Li et al., 2000). These references must not be cited as Li et al. (2000a,b).

• References are cited in the text in increasing chronological order (but all references by the same author(s) are given together) and alphabetically when published in the same year (Brown, 1988, 2003; Brown & Smith, 1989; Smith & Brown, 1989, 1991; Brown, Smith & Jones, 1990; Brown et al., 1991; Jones, 1999a,b).

• Reports and monographs from previous JECFA meetings are cited in the text as “(Annex 1, reference xxx)” and are not included in the reference list. Annex 1 refers to the list of previous JECFA publications that is included at the back of both the meeting report and the publication containing all of the toxicological monographs from the meeting.

• Personal communications and other unpublished information are cited in the text only, not in the reference list. They should be cited as follows: [name of authority cited], [name of institution], unpublished data or unpublished observations or personal communication, [date]).

• For information on the formatting of references for the reference list at the end of the monograph, see section 2.3.5.

2.2.8 Miscellaneous

• Monographs should be concise documents, with only as much detail as is necessary to be able to understand and reproduce the evaluation; too much detailed description of irrelevant studies and too many non-critical tables should be avoided. Monographers need to make every effort to reduce the length of their monographs without eliminating essential information.

• The physical meeting is referred to as “the meeting” (e.g. the meeting was held in April); the group of meeting participants is referred to as “the Committee” (e.g. the Committee established an ADI of 0–0.3 mg/kg bw).

• “JECFA” is referred to, rather than “the JECFA”. Reference to previous Committees should be made by number (e.g. the thirty-sixth meeting of the Committee) rather than by year, because in many cases reports were not published in the same year as the meeting and in some years more than one meeting was held, which creates confusion.

• It is conventional to list countries alphabetically, and country names must correspond to the most current listing of Member States and Associate Members of WHO, as given in the
current version of the WHO style guide or an interim updated list of Member States and Associated Members of WHO.

- Where the POD is a NOAEL, this is "identified", as it is one of the dose groups used – for example, "0.5 mg/kg bw per day was identified as the NOAEL". Health-based guidance values (ADI, ARfD) are "established" – for example, "the Committee established an ADI of 0–0.1 mg/kg bw".

- Within each toxicological section (short-term studies of toxicity, long-term studies of toxicity and carcinogenicity, etc.), studies should be presented in order of species size, from smallest to largest (this differs from many other organizations, where the rat is presented before the mouse). Headings for each species should be included when more than one species is discussed ((a) Mice, (b) Rats, (c) Hamsters, (d) Rabbits, (e) Dogs, (f) Pigs, (g) Monkeys), but no species heading is necessary (although it can be inserted if desired) if only one species is discussed. It should be noted that "monkeys" comprise a higher phylogenetic grouping than species, and the individual species (e.g. cynomolgus, rhesus) should be specified.

- Each study summary should provide a short description of the methodology used in the study. Most studies will comply with an Organisation for Economic Co-operation and Development (OECD) test guideline or equivalent national guideline, and in such cases there is no need to provide lengthy descriptions of the methodology. Attention should be drawn to any deviations from the test guideline, either omissions or significant additions. If in-life examinations such as ophthalmoscopy and blood sampling are performed at multiple time points, these time points should be identified. This is particularly useful when there is a need to establish an ARfD, as such early measurements in repeated-dose studies may provide the critical effect for such a health-based guidance value.

- Studies performed before the implementation of good laboratory practice (GLP) will be considered on a case-by-case basis, with careful consideration of the quality and appropriateness of the study.

- JECFA style is to use free-flowing text rather than large numbers of subheadings for each particular level of investigation.

- Overall conclusions of the Committee regarding, for example, the carcinogenicity and genotoxicity of a compound should generally be reserved for the Comments section. Conclusions of the Committee regarding specific studies (e.g. when the Committee disagrees with the study authors' conclusions) are given in the body of the monograph.

- WHO house style uses a mix of British and North American spellings. Examples of spellings of some commonly used words in JECFA monographs are as follows: anaesthetize, analyse, antimicrobial, caesarean, centre, coenzyme, colour, cooperate, criticize, decision-making, diarrhoea, end-point, estrogen, et al., etiology, faeces, feed (for animals, not food), fetus, haemoglobin, homepage, hypocalcaemia, in vitro, in vivo, leukocyte, litre (L, not l), meta-analysis, metabolize, modelled, neurobehavioural, oedema, oesophagus, oxidize, paralyse, pharmacopoeia, postmortem, postnatal, postpartum, pretreatment, programme, re-examine, reopen, side-effect, subgroup, sublethal, sulfur, tumour, webpage, website, worldwide, X-ray.

- Abbreviations are defined the first time they are used in the text; thereafter, only the abbreviation is used. A list of abbreviations should be prepared for each monograph. This list will be incorporated by the editor into an overall list of abbreviations for all monographs published after the meeting.

- Monographs will be edited according to the most recent version of the WHO style guide. Monographers can request a copy of the WHO style guide from the WHO Joint Secretary.

**2.3 Detailed content of the monograph**

The guidance in this section approaches the content of the monograph from the viewpoint of the end result of the meeting – that is, the production of the report item.

As mentioned above, the monograph should contain sufficient information to permit all the details required for the report item to be identified and independently confirmed. If a monographer is in doubt
about whether to include extra detail, it should be added to the monograph so that it is available for others to see; it can always be deleted following discussion at the meeting.

Until the final changes are made by the monographer at the end of the meeting, the monograph is a draft document to support the discussion. It is therefore often helpful to include comment boxes or highlighted text that draws attention to potentially important or contentious aspects of the evaluation, as long as these are subsequently deleted. It is important for the monographer to recognize that the final monograph is the product of the Committee and not of the monographer.

If no studies were available for one of the main headings in the template, this should be noted in the monograph.

For monograph addenda, it may not always be sufficient just to consider new data, especially if there have been changes in evaluation criteria since the last evaluation (e.g. check for findings early on in studies that might be relevant to establishing an ARfD). An appropriate description of any such studies that were considered in the present evaluation should be included in the monograph addendum. Sometimes, it may be sufficient to copy and paste relevant sections from the previous monograph, in which case they should be so indicated (e.g. indented, italics or smaller font).

Details that should appear in each section of the monograph are described in the following sections. Note that the section numbering used for the headings (shown in red) is that used in the actual monograph (see also Annex 1). A table of contents generated from the final headings used in the monograph should be included on the first page of the monograph, below the title, authors and authors' affiliations. All individuals contributing to the preparation of the draft monograph for the meeting should be listed as authors. As the end product reflects the discussion of the Committee at the meeting, the listing of authors is preceded by the phrase “First draft prepared by”.

2.3.1 Explanation

1. Explanation

- This part of the monograph will form the basis for the first few paragraphs of the report item. The editor will insert the final version of the Explanation section (following the adoption of the meeting report) into the final draft of the monograph. The monographer can indicate whether any detailed information that was deleted from the Explanation section during the preparation of the report item should be retained in the final monograph.

- The first paragraph should provide information on the identity of the veterinary drug (e.g. Chemical Abstracts Service [CAS] registry number, common [International Nonproprietary Name (INN) or International Organization for Standardization (ISO)] and/or chemical [International Union of Pure and Applied Chemistry (IUPAC)] names, production, composition, etc.).

- The second paragraph should briefly describe the veterinary drug’s approved uses, including route of administration, species in which it can be applied and approved doses. The veterinary drug’s mechanism of action can be briefly described.

- The third paragraph describes whether the Committee has previously evaluated the veterinary drug. If so, the number of the meeting at which the previous evaluation (or evaluations) was performed is indicated and referenced by number using the standardized reference list of JECFA publications, which may be found in Annex 1 of recent JECFA reports (WHO Technical Report Series) and toxicological evaluations (WHO Food Additives Series). Thus, the report of the forty-third meeting would be referenced as (Annex 1, reference 113), and the toxicological monographs prepared after the forty-third meeting would be referenced as (Annex 1, reference 114). Reasons for the present re-evaluation should be given, and, if a full monograph on a substance that has been evaluated previously is being prepared, a statement should be made to the effect that the previously published monograph has been expanded and is reproduced in its entirety below.

- If the veterinary drug has previously been evaluated by JMPR for pesticidal use, the date and number of the meeting (or meetings) should be provided. The reference(s) for the meeting should be cited using the author/date system (e.g. FAO/WHO, 2014 for a meeting report and WHO, 2014 for toxicological monographs).
If the Committee has not previously evaluated the veterinary drug, the reason for it being placed on the agenda should be given. For example, “[Veterinary drug X] has not previously been evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The Committee evaluated [veterinary drug X] at the present meeting at the request of the [Xth] Session of the Codex Committee on Residues of Veterinary Drugs in Foods (FAO/WHO, 20xx) with a view to establishing an acceptable daily intake (ADI) and recommending maximum residue limits (MRLs) in [animal species] tissues.”

A figure illustrating the chemical structure of the veterinary drug should be included in the full monograph (but not in the report item).

The final paragraph of the Explanation summarizes the type of data that were provided by the sponsor, whether the critical studies were conducted in compliance with GLP, unless otherwise specified, and whether the overall package was considered sufficient to establish a robust ADI. In addition, whether a literature search was conducted, the keywords used and the number of references obtained should be provided. If the search strategy is complex, it might be more appropriate to describe this in an appendix.

FAO prepares its own Explanation section. If at all possible, the WHO and FAO monographers should attempt to merge their two versions of the Explanation section prior to the meeting. During the meeting, the merged version could be revised following discussions by both groups. If this is not possible, the WHO and FAO counterparts for each veterinary drug should make every effort to merge their two versions of the Explanation section during the meeting. As a last resort, the editor will merge the two versions after the meeting and obtain approval from both the WHO and FAO monographers.

2.3.2 Biological data

2. BIOLOGICAL DATA

Biological data should be grouped under three headings: Biochemical aspects, Toxicological studies and Observations in humans.

In a full monograph, but not in a monograph addendum, if no data are available under any of these headings or under subheadings under Toxicological studies, except for Special studies, the heading should be included in the monograph together with the statement “No information was available”.

2.1 Biochemical aspects

2.1.1 Absorption, distribution and excretion

Information in this section should be restricted to experimental animals, such as mice, rats, rabbits and dogs. Information on pharmacokinetics in food-producing animals, such as cows, goats, horses and poultry, will be covered by FAO in the Residue evaluation section of the meeting report and in the FAO residues monograph and should not normally be repeated here. Information on pigs can be included if the pig is considered to be a good animal model for humans for the end-point of concern; this will be determined on a case-by-case basis.

Information on metabolism should not be included in this section. Rather, it is included in section 2.1.2 on biotransformation.

For each study, details on the position and type of any radiolabel used, test species, sex and number of animals, dose levels used (in terms of both the drug [mg/kg bw] and radioactivity [MBq/kg bw], as appropriate) and route of exposure should be provided.

Information in this section includes, where possible:

- hydrolysis/metabolism of the parent compound and its products in the mammalian gastrointestinal tract (including products of metabolism by the gut microflora) (distinguish between hydrolysis/metabolism before absorption and of biliary excretion products);
- Rate and extent of absorption of the unchanged compound and its hydrolysis or digestion products, with time to maximum concentration ($T_{\text{max}}$) and the concentration achieved ($C_{\text{max}}$);
- Bioavailability of the parent compound;
- Pattern and rate of distribution of absorbed substances to tissues and organs within the animal;
- Mode, rate, and extent of excretion or elimination of the parent compound and/or radiolabel from blood and tissues, with percentage recovery in major excreta (urine, faeces, bile) over a given time interval (e.g., 35% in urine from 0 to 48 hours);
- Pharmacokinetic parameters, such as volume of distribution, terminal elimination half-life from plasma and total body clearance.

- It should be made clear as to whether the findings relate to the radiolabelled material or to the parent compound.
- Differences between sexes, dose sizes and single versus repeated dosing should be noted, together with any other relevant findings.

### 2.1.2 Biotransformation

- Information in this section should be restricted to experimental animals, such as mice, rats, rabbits, and dogs. Information on metabolism in food-producing animals, such as cows, goats, horses, and poultry, will be covered by FAO in the Residue evaluation section of the meeting report and the FAO residues monograph and should not be repeated here. Information on pigs can be included if the pig is a good animal model for humans for the end-point of concern; this will be determined on a case-by-case basis.
- Where information on metabolism has been obtained from studies previously described in section 2.1.1, a brief study description and cross-reference to that section can be made, rather than repeating full study details.
- This section describes the metabolism of the parent compound, if absorbed as such, and of its products if they are not normal dietary or body constituents. Information on the main routes of metabolism, the metabolite profile and the mode, rate, and extent of excretion or elimination of identified metabolites is included here.
- A biotransformation or metabolic scheme showing the main metabolic reactions should be included along with an identification of the species to which it applies. Such schemes are usually provided by the sponsor and can be scanned for inclusion in the monograph. If the data sponsor does not provide a metabolic scheme, it should be requested.

### 2.1.3 Effects on enzymes and other biochemical parameters

- This section describes the effects of absorbed substances and/or their metabolites on cellular and tissue enzyme production and morphology, chemical constitution, enzyme activity or physicochemical state.
- If there is no relevant information to be included in this section, the heading can be deleted.

### 2.2 Toxicological studies

- This section contains summarized descriptions of toxicological studies that are important for assessing the safety of the substance. These summaries generally comprise the bulk of the monograph. Information from five main categories of studies on veterinary drugs should be routinely included: Acute toxicity, Short-term studies of toxicity, Long-term studies of toxicity and carcinogenicity, Genotoxicity, and Reproductive and developmental toxicity. Sometimes these routine studies point towards the need to look at particular target organs or tissues or end-points; such studies are classified as Special studies.
• Studies that provide the basis for the evaluation should be summarized in greater detail than other studies. Single paragraphs composed of one-sentence summaries may be sufficient for reporting the results of studies of limited design or minor relevance for the evaluation.

• The study conclusions should be summarized in this section. If the person who arrived at the conclusion is not identified, it is assumed that it is the author(s) of the study and that the monographer agrees with the conclusions. When the monographer disagrees with the conclusions of the study author(s), he or she should discuss the contentious issues and present his or her own conclusions as a separate paragraph, to flag the issue for discussion by the Committee. In the final monograph, the paragraph concludes with the Committee’s conclusion and identification of the NOAEL and the study reference.

• When adjacent paragraphs summarize different studies under the same heading, an extra space should be left between them.

• The GLP status of the study, along with the relevant authority, should be indicated. If there is no GLP certification, the monographer should at least note whether the study was inspected by a quality assurance (QA) unit, as noted by the presence of a signed QA statement, and make some comment on the apparent quality of the protocol and adequacy of the methods used. In addition, whenever the author provides information on the test guideline or protocol that was followed, it should be so indicated.

• General study details that should be provided for each toxicological study described in the monograph include the following:
  
  o purpose or objective of the study;
  o identity, specification and purity of the test material and its batch and/or lot number;
  o species and strain of animal used;
  o the method of dosing (e.g. gavage, capsule, variable dietary concentration);
  o vehicles used for gavage studies (and if there appear to be any findings that change with different vehicles); if the vehicle is not provided, it will be assumed to be water;
  o purity of the test material and its batch and/or lot number, and the nature of any potentially toxicologically important impurities;
  o sex and number of animals in each group (if there are satellite groups, numbers for the main group and satellites are given separately);
  o whether a study is non-guideline or a range-finding study with limited investigations. In such cases, a conclusion on the value of the study in evaluating the toxicological profile of the compound (e.g. provides useful information on repeated-dose effects; end-points studied too limited to provide useful information) can be provided;
  o any additions to the standard test protocol, such as measurement of specified hormone levels or evaluation of toxicokinetics during the dosing period;
  o all the administered dose levels, including 0 for controls; for dietary studies, this should include both mg/kg feed values and the equal (if measured) or equivalent (if based on dose conversion factors) mg/kg bw per day dose for both males and females; for drinking-water studies, this should include both mg/L drinking-water values (even if originally given as parts per million) and the equal (if measured) or equivalent (if based on dose conversion factors) mg/kg bw per day dose for both males and females. If the author of a study presents administration levels in terms of mg/animal per day, these values should be converted to mg/kg bw per day using animal weights if they are included in the report;
  o whether the study used dose patterns that did not involve dosing every day (e.g. 5 days/week rather than 7 days/week). If so, it should be checked whether the stated dose levels are given only for the days of dosing or averaged over the whole duration of the study. JECFA gives dose levels averaged over the entire study duration;
  o whether there were any complications associated with dosing, such as solubility, stability and palatability;
  o duration of the study;
  o details of any recovery group (e.g. numbers, duration of dosing, interval between last dose and termination, extent of investigation of animals in this group);
  o any mortalities seen during the study, both in treated and in control groups, and information on the causes of mortality, if known (e.g. dosing errors in gavage studies, which are not compound related);
o description of compound-related findings, if any, identifying the effect, its severity or magnitude and, for dichotomous data, an indication of the number of animals affected. This is often presented in tabular form. If no significant effects were seen at a particular dose level, a simple statement to that effect should be made;
o whether there is dose-dependency of the findings and if not, whether there is any explanation for this;
o relevant information on historical control data, if available, where this may help in the interpretation of the findings (e.g. marginal effects at highest dose, incidence in controls is particularly high or low);
o any findings that are statistically significant but are discounted as not adverse or not relevant to a human risk assessment;
o any in-life findings early in the study that might be relevant to establishing an ARfD (e.g. body weight changes or behavioural effects after one or a few days of dosing);
o anything else of note in the study (e.g. high morbidity in controls);
o the POD, such as the NOAEL (if one was identified), and the critical findings on which the POD was based (e.g. at the LOAEL), given at the end of the study description;
o the author(s) and date of preparation of the study report, given at the very end of the study description (even if already cited at the beginning of the study description).

- Where relevant information is identified in the peer-reviewed literature that provides insight into the toxicity of the compound additional to that provided by the studies submitted by the sponsor, it should be summarized under the appropriate heading below. In general, the same format should be followed as for a submitted study, highlighting any important details that are missing (e.g. details of dosing regimen) and also any observations additional to those that might be found in a typical guideline study, which may be of value in evaluating the compound.

2.2.1 Acute toxicity

- Acute toxicity studies can provide useful information regarding target tissues and species and sex differences.

- The results of acute toxicity studies that are expressed in terms of the median lethal dose (LD_{50}) (used for oral, intramuscular, intraperitoneal or dermal administration) and/or median lethal concentration (LC_{50}) (used for administration by inhalation) should be presented in tabular form, as shown in Annex 2.

- When three or more LD_{50} or LC_{50} determinations by the same route in the same species are available, the results may be expressed as a range in which the lowest to the highest values are recorded.

- Other acute toxicity data important to the evaluation, such as the nature of toxicity, clinical signs and target tissues, may be presented in summary form as text or in table notes below the table.

- Although acute dermal and inhalation studies, dermal and ocular irritation studies and dermal sensitization studies are not directly relevant to the work of JECFA (i.e. dietary risk assessment purposes) in the majority of cases, these studies can in some cases provide useful supplementary information to assist with the hazard assessment (e.g. confirmation of localized irritation of the gastrointestinal tract by observations of skin and/or eye irritation). They can also increase the utility of the monograph for use by others. On this basis, a concise summary of the results of these non-oral toxicity studies will generally be included in the monograph, where available.

- The dermal and ocular irritation studies are described briefly, with no or minimal methodological details, although the species in which the studies were performed should be indicated.

- The dermal sensitization description should include the numbers of any animals responding and the test method used.
2.2.2 Short-term studies of toxicity

- Toxicological studies in which substances are administered in regularly repeated doses over periods ranging up to, but not including, one year for most small animal species and up to two years for dogs and primates should be summarized in this section.

- These studies, when properly performed, provide important information regarding the major toxic effect(s) of the test substance and its dose–response relationships. Short-term studies of toxicity are often performed to help in dose selection for long-term studies of toxicity, and they can give some indication of target tissues and organs. In some cases, short-term studies of toxicity can help clarify lowest-effect dose levels for effects observed in long-term studies of toxicity, and they can provide information that is useful for the interpretation of long-term studies of toxicity and carcinogenicity (e.g. early signs of toxicity in the kidney or liver when tumours appear in these organs after long-term exposure).

- It can be useful to comment on findings that were not seen in a study if they were seen in another similar study. For example, if a certain effect was seen in a 28-day rat study, it would be expected that it would also be present in the 90-day study at similar, or lower, dose levels.

- Any findings that were considered not relevant to humans and the reasons why (e.g. kidney findings in male rats only, supported by investigations of $\alpha_2$-microglobulin) should be indicated.

- Any early findings of possible relevance to an ARfD, even if at doses above the LOAEL, should be identified, together with the time and the lowest dose at which they were seen. When effects are observed after a few days of dosing, but this was the first time of observation, this should be clearly stated.

2.2.3 Long-term studies of toxicity and carcinogenicity

- Toxicological studies in which substances are administered in regularly repeated doses in feed or drinking-water over the greater part of the normal lifespan of the animal species (i.e. one year or longer for most small animal species, in line with OECD test guidelines, and two years or more for dogs and primates) are summarized in this section. These studies are used for detecting chronic effects that are not observed in shorter-term studies or that show progression with duration of dosing. Long-term studies that are designed to investigate specific effects, such as carcinogenicity, should be included in this section. Often long-term studies, particularly in rats, are designed to assess both chronic toxicity and carcinogenicity.

- Because certain animal strains have high background levels or susceptibilities to developing certain tumour types, it is important to give the strain details.

- If the nature of the dose–response relationship is not clear (e.g. response is marginal and is not monotonic) or there is concern about the incidence level in the controls, historical control data should be provided. These should be requested from the sponsor, if necessary.

- It should be indicated whether the survival rate is adequate in the top-dose animals (there should normally be a minimum of 25 animals [50% of a standard group size of 50] in each group surviving to termination). If survival did not meet this level, it should be indicated whether the deaths were mainly towards the last few weeks of the study and whether survival was adequate to enable the various end-points in the study to be assessed.

- It should be noted whether there is any indication of findings occurring earlier in treated animals. This can be important for lesions that have a high background incidence and in interpreting some long-term effects.

- General study details that should be provided for each toxicological study are given above. In addition, the types of observations made (e.g. mortality, feed and water consumption, body weight, haematology, clinical chemistry, urine analysis, ophthalmoscopy examinations, physical/neurological examinations, functional observational batteries, clinical signs, organ weights, gross pathology and histopathology) and any other information about the design of the study considered to be noteworthy should be provided. When histopathological examinations were performed, the tissues that were examined should be indicated, along
with the identification of tissues that were of particular interest to the evaluation and whether only certain dose groups were investigated.

- Negative findings should be limited to general statements on survival, growth, organ weights, tumour incidence, organ function tests, and gross and microscopic appearance of tissues. In particular, if there were no compound-related increases in tumour incidences, a clear statement should be made.

### 2.2.4 Genotoxicity

- Data from an appropriate range of in vitro and in vivo genotoxicity tests can be useful in elucidating the mechanism of toxicity of certain compounds. The results of these studies are also considered when evaluating the results of rodent carcinogenicity bioassays and when determining whether an in vivo carcinogenicity bioassay was necessary to enable adequate assessment of the carcinogenic potential of a substance.

- To present the data in a more understandable form and to conserve space, the results of genotoxicity tests should be tabulated. Annex 2 provides examples of the tabular representation of such data.

- Where the results of a particular genotoxicity study were considered positive or equivocal (e.g. colony counts, size of colony, survival rates or aberrant cell numbers), the study can be described in more detail in textual form or in table notes below the summary table.

### 2.2.5 Reproductive and developmental toxicity

#### (a) Multigeneration reproductive toxicity studies

- Multigeneration reproductive toxicity studies provide general information on the effects of the test substance on gonadal function, estrous cycles, mating behaviour, conception, parturition, lactation, and growth and development of the offspring until the age of weaning.

- With dietary exposures at constant milligram per kilogram feed concentrations, the achieved intakes vary greatly with reproductive stage. Achieved intakes are normally determined for various stages of the study (e.g. premating, lactation). When determining NOAELs, JECFA policy is to use the lowest achieved intake of any of the measured stages, unless a critical stage and associated intake can be determined.

- Data should be presented for each stage and generation separately (e.g. parental generation for first generation, first mating pups, parental generation for second generation, etc.). Some indication of whether findings were consistent across the generations should be provided subsequently.

- It should be indicated whether litters were standardized in size at around day 4.

- If pup weights are different in treated groups, it should be determined if this relates to litter size and if there are effects on total litter weight.

- If pup mortality is increased in treated groups, it should be determined if there were more pups in the litters to start with (e.g. control litter mean of 10.8 pups with 0.9 dying gives 9.9 alive; test group mean of 12.1 pups with 2.1 dying might be statistically significant, but still gives 10.0 alive, more than in controls).

- For developmental end-points (e.g. tooth eruption), it should be determined if there are effects on the time to achievement; the body weight at that time should also be checked.

- PODs, usually NOAELs, should be identified for reproductive toxicity (e.g. impairment of fertility, parturition, lactation), parental toxicity (usually systemic toxicity, such as effects on body weight or feed consumption) and offspring toxicity (e.g. effects on pup body weights or pup viability).
(b) Developmental toxicity studies

- Developmental toxicity studies are used for assessing effects on the developing organism, which may include death of the developing organism, structural abnormalities, altered growth or functional deficiencies.
- The days of dosing should be indicated (i.e. which days of gestation).
- Details of the investigative techniques (e.g. dissection, staining, X-ray) and the proportion of fetuses being examined by each technique should be given.
- If a range-finding study has been submitted, it can be described separately if there are important findings. If it adds nothing to the discussions, it can just be mentioned in the introduction to the main study.
- The unit for statistical comparison in developmental toxicity studies is the litter, not the individual fetus. Hence, when statistically significant differences are reported in incidences relative to the total number of fetuses per dose group, the monographer should check whether such differences are also apparent when the results are expressed per litter.
- If there are developmental anomalies in the main test, it should be determined if they were seen in the range-finding study as well (if submitted). Range-finding studies normally include only limited examinations of maternal toxicity, external malformations and fetal viability.
- It should be indicated whether there were any increases in malformations, even at maternally toxic doses.
- Any effects that occur within the first day (or first few days, if this was the first time of observation) after dosing should be noted, as they could be used as the basis for establishing an ARfD.
- PODs, usually NOAELs, should be identified for maternal toxicity (usually systemic toxicity, such as effects on body weight or feed consumption) and for embryo and fetal toxicity (e.g. effects on fetal weight, fetal mortality, incidence of skeletal and visceral anomalies or variants).

2.2.6 Special studies

- Special studies, when relevant and submitted by the sponsor in support of the safety evaluation of the compound or identified from a literature search, should be reviewed.
- It is important for the monographer to be aware that special studies do not typically follow specific well-established protocols, but rather are designed to resolve particular scientific issues and concerns, and protocols may vary from study to study; hence, with the exception of residues of veterinary drugs with antimicrobial activity, no specific guidance is included here.
- General study details reviewed and described by the monographer might include the objective of the special study, number, species and strain of animals, any details specific to the unique special study, the study outcome and its relevance to the end-point being investigated.
- Examples of the types of special studies that might be included are special studies on cardiovascular effects, immune responses, macromolecular binding, metabolites (the toxicological importance of the metabolites identified in section 2.1.2 on biotransformation should be discussed, if known), no-hormonal-effect levels, ocular toxicity, photoisomerization products, relay toxicity (toxicity of incurred residues in feed from animals treated with the veterinary drug), thyroid function and neurobehavioural effects.
- Studies on the pharmacological effects of veterinary drugs may be included, particularly when such an effect is the primary mode of action for its veterinary use (e.g. β-adrenoceptor activity). A variety of systems are used to conduct these studies, including computational structure–activity or docking models, isolated and/or recombinant expressed proteins, in vitro cell systems, ex vivo tissue or organ preparations and in vivo systems. Suitable details on experimental design, concentration– or dose–effect relationships, potency, whether the drug is an agonist or antagonist, and specificity (e.g. for receptors and receptor subtypes) should
be provided. Any factors influencing potency should be noted. Information on species comparisons of specificity and potency can be very helpful in evaluating the compound.

- Special studies should be listed alphabetically.

### 2.3 Microbiological effects

- Microbiological effects of veterinary drugs have in the past been considered in the subsection on *Special studies* in the section on *Toxicological studies*. However, given that both a toxicological ADI and a microbiological ADI may be derived for the same compound, it was concluded that it would be preferable for microbiological effects to be described in a separate section rather than under *Toxicological studies*.

- The information to be included in this section is described in detail in Chapter 4.

### 2.4 Observations in humans

- Observations in humans can be particularly useful for establishing health-based guidance values, for assessing the relevance of the results of studies in experimental animals and for confirming health-based guidance values.

- All studies dealing with humans (except for those summarized under *Biochemical aspects* or *Microbiological effects*) should be included in this section, including epidemiological surveys, clinical experience (some veterinary drugs may have been investigated for use as therapeutic agents in humans), anecdotal observations, health effect studies relating to occupational exposure, reports of abuse, and volunteer studies measuring pharmacological effects or intolerance.

- Details such as numbers of subjects, sex, age and general statements of physical condition should be given if important to the evaluation.

- JECFA will use human data in establishing health-based guidance values if the study is scientifically valid and performed ethically, according to the principles of the Declaration of Helsinki. Documentary evidence of this should be provided by the sponsor, or, where the results are from a published study, a statement to this effect should be included in the paper.

### 2.3.3 Comments

### 3. Comments

- The objective of the *Comments* section is to provide a concise summary of the relevant biochemical/toxicological/microbiological information and its interpretation, while providing sufficient explanation that the bases for the conclusions of the Committee are clear. This section should contain short summaries of the biological findings in the studies of significance for the evaluation. The findings should be listed in the same general order as they are summarized in the main body of the monograph under the headings *Biochemical data*, *Toxicological data* and *Microbiological data*. Short paragraphs that consist of relevant findings in individual studies should be used instead of long paragraphs that include results from several studies.

- The *Toxicological data* section will usually begin with the statement: Critical studies relevant to the risk assessment are summarized in Table X, followed by a summary table. This statement will be deleted from the meeting report, and the summary table will be moved to the end of the report item (see Chapter 3).

- An introductory paragraph that identifies the critical effects in the overall database can precede the individual study descriptions.

- All relevant NOAELs (or other PODs) should be included. Only one value for the NOAEL in units of mg/kg bw per day (usually for the sex with the lower value, except when the critical effect is sex specific) should be given for each study.
• Details such as sex, age and general statements of physical condition should be given if important to the evaluation of observations in humans.

• Strains of animal species are not provided in this section unless critical effects are known to be strain specific.

• If there are no microbiological effects of concern, a standard phrase should be added under the Microbiological effects heading (see Annex 5).

• The Comments section will comprise the bulk of the meeting report item (see Chapter 3).

• Conclusions of the Committee regarding the carcinogenicity, genotoxicity, teratogenicity and neurotoxicity of each compound should be drawn using the standard phrases given in Annex 5.

• All statements in the Comments section should be referenced. This is a change from the usual practice, but it makes the Committee’s conclusions more open, transparent and verifiable and enables the Comments section to be more readily used by other interested groups.

• Annex 3 contains a template for the report item, and Annex 4 provides two examples of report items. Both should serve as a model for the preparation of the Explanation (see section 2.3.1), Comments and Evaluation sections (see section 2.3.4).

2.3.4 Evaluation

4. Evaluation

• A proposal should be provided by the reviewer as to whether the Committee should establish one or more health-based guidance values (i.e. an ADI or an ADI and an ARfD) and, if so, on what basis.

• The health-based guidance value can be established on the basis of toxicological (including pharmacological) or microbiological end-points.

• If the health-based guidance value is established on the basis of a toxicological end-point, the NOAEL (in mg/kg bw per day), the effects on which the NOAEL is based, the study from which the NOAEL has been identified and the safety or uncertainty factor applied to the NOAEL should be provided.

• The health-based guidance value may be established on the basis of the POD (e.g. the NOAEL) from one study or from more than one study, when the PODs are identical or very similar. Additional PODs from other studies may also be used to support the health-based guidance value – for example, when the POD is similar to the critical POD but the study was not as comprehensive.

• EHC 240 (IPCS, 2009) and previous JECFA reports on residues of veterinary drugs should be consulted for guidance on the selection of safety factors in establishing ADIs. If a safety factor other than 100 is used, the reason must be stated.

• For compounds that have effects on microbiological end-points, the health-based guidance value based on toxicological effects is designated the toxicological health-based guidance value and is not established until completion of the microbiological evaluation.

• If the compound has effects on a microbiological end-point that is more sensitive than the toxicological end-point, the health-based guidance value is based on the microbiological effect. Whether a microbiological ADI is derived from a minimum inhibitory concentration (MIC) or a no-observed-adverse-effect concentration (NOAEC), an uncertainty factor is generally not required. However, an uncertainty factor between 1 and 10 may be considered, depending on the quality and quantity of the data (IPCS, 2009; VICH, 2012).

• The health-based guidance value is established as the lower of the upper bound of the toxicological ADI (or ARfD if appropriate) and the upper bound of the microbiological ADI (or microbiological ARfD if appropriate).
• If the compound produced severe or irreversible effects (e.g. carcinogenicity by a non-genotoxic mode of action), the margin between the upper bound of the ADI and the LOAEL for the severe effect should be given (see also Chapter 4).

• The ADI is expressed as a range from 0 to \( x \) mg/kg bw (not mg/kg bw per day), and the upper bound of the range \( (x) \) should be rounded to one significant figure.

• If a temporary ADI\(^6\) is proposed, information required on the substance should be listed, along with a date by which time the results of the indicated studies should be submitted to WHO for evaluation.

• If no ADI can be established because there were critical data gaps or because of appreciable biological uncertainty in the assessment, the information that the Committee would wish to have before reviewing the compound again should be listed.

• If a veterinary drug that has a temporary ADI is being re-evaluated, the Committee has the option to (1) remove the temporary designation and establish an ADI, (2) extend the temporary ADI or (3) not extend the temporary ADI (i.e. the ADI is withdrawn).

• For veterinary drugs that are mixtures, a group ADI may be established (e.g. streptomycin/dihydrostreptomycin, enrofloxacin/ciprofloxacin).

• An ADI “not specified” (see Annex 1 in EHC 240; IPCS, 2009) may be established for a veterinary drug where there is a large margin of safety for consumption of the residues in food when the drug is used according to Good Practice in the Use of Veterinary Drugs and it is not considered necessary to establish a numerical ADI.

• The ARfD, if established, is given as a single number (\( x \) mg/kg bw).

• The critical effect for establishing an ARfD might be applicable to only a specific subpopulation. Hence, it could be appropriate to establish such an ARfD only for the at-risk subpopulation. The most common instance of this would be developmental effects. Only pregnant women would be at risk from acute exposure to such a compound, and hence the ARfD might be established for women of childbearing age (as a surrogate for pregnant women). Where acute systemic effects also occur, albeit at higher doses, but still of potential concern to consumers, a second ARfD could be established for the general population.

• A statement should be included on the possibility, if any, for refinement of the ARfD – for example, when it is based on effects seen after several days of dose administration, because this was the first time point for observations.

• If an ARfD is not established, a statement should be added explaining why such a health-based guidance value is not needed. For example: The use profile of [compound x] as a veterinary drug is such that dietary exposure to [compound x] from a large portion is unlikely to be markedly greater than that from chronic consumption. The toxicological profile of [compound x] is such that it is unlikely to present an acute hazard. The Committee therefore concluded that it was not necessary to assess the acute risk from exposure to [compound x] when used as a veterinary drug.

2.3.5 References

5. References

• References should be presented at the end of the monograph; the sponsor should be asked to provide the references in the JECFA style.

• In the reference list itself, all authors should be given if there are six or fewer; if there are more than six authors, the first six authors are given, followed by et al.

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\(^6\) Used when data are sufficient to conclude that use of the substance is safe over the relatively short period of time required to generate and evaluate further safety data, but are insufficient to conclude that use of the substance is safe over a lifetime. A higher than normal uncertainty or safety factor is used when establishing a temporary ADI, and an expiration date is established by which time appropriate data to resolve the safety issue should be submitted for evaluation. The temporary ADI is listed in units of milligrams per kilogram of body weight.
• Order of references in reference list: single author by increasing year, two authors alphabetically by second author, three authors alphabetically by second or third author, more than three authors by increasing year:


• Abbreviated journal names as given by the United States National Library of Medicine (e.g. Am J Toxicol) are used. Note that the abbreviated journal name ends with a period, before the volume number.

• Page ranges use en dashes and are abbreviated (only those digits that change in the higher page number are given): e.g. 310–7; 252–66; 296–305.

• Examples of references in reference list:

Journal reference

Book reference

Unpublished study
There is room for flexibility in the format of unpublished studies. The essential elements of unpublished studies that should be included, where available, are:

○ the name of the author(s) who performed the research work, if provided;
○ the year in which the experimental work was completed;
○ the title of the experimental study (if the title is in a language other than English or French, translation of the title into English is preferred);
○ study number, if provided;
○ an indication that the study is unpublished;
○ the name of the institution at which the experimental study was performed;
○ the name of the institution that submitted the report to WHO.


Reference in a foreign language (other than French)

Secondary reference

Example:

Reference found online
Give URLs, with access dates, for as many references as possible, particularly WHO products.

Example:

Databases, electronic publications and website references


Section of a website

Online journals
During the first few days of the meeting, the Committee will discuss in detail each veterinary drug monograph, resolving any contentious issues raised by the monographer and reaching agreement on the general approach to be taken in evaluating the veterinary drug. Once agreement has been reached on a way forward, the monographer should prepare the first draft of the meeting report item, based on the Explanation, Comments and Evaluation sections in the monograph.

The monographer prepares the report item during the meeting by following the template shown in Annex 3 (the current template will be made available on the computers in the meeting room). This involves extracting the Explanation, Comments and Evaluation sections from the monograph into the meeting report template and modifying them as suggested during initial discussions of the Committee. In addition, the monographer must prepare a Summary and conclusions section for the report, which consists primarily of the summary table prepared for the Toxicological data section of the Comments, as well as some additional summary material.

The Explanation section prepared by the WHO monographer should be merged with the Explanation section prepared by the FAO counterpart during the meeting, if this has not already been done prior to the meeting. If this cannot be done due to time constraints, the editor will merge the two sections after the meeting and submit them for approval to the WHO and FAO authors.

The Evaluation section in the report ends with a statement that a toxicological monograph or monograph addendum has been prepared. This statement is deleted in the monograph.

The Summary and conclusions section begins with a table entitled “Studies relevant to risk assessment.” This table contains only studies and NOAELs/LOAELs (or other PODs) critical to the conclusions used in the risk assessment. For dietary studies, the NOAELs and LOAELs are given as mg/kg bw per day only. The table will normally include long-term toxicity and carcinogenicity studies in mice and rats; reproductive toxicity studies in rats and developmental toxicity studies in rats and rabbits; repeated-dose toxicity studies in dogs; and any studies used as the basis for, or to support, the establishment of the ADI or ARfD. The table does not normally include repeated-dose toxicity studies of 90 days’ or shorter duration in rodents, unless used to establish the ARfD or ADI. Footnotes to the table indicate where the NOAEL is the highest dose tested or the LOAEL is the lowest dose tested. An additional footnote is used to indicate when the NOAEL has been obtained from two or more studies combined. The pivotal study is also bolded and indicated in a footnote, together with the study reference.

The Summary and conclusions section includes contributions from both FAO and WHO experts and will need to be merged during or after the meeting.

References are cited for all studies in the report item. The editor will retain only the references for the key studies cited in the text or tables and change them to italicized numbers for the meeting report. This facilitates the use of the meeting report item for the final monograph, in which the Comments section is fully referenced.

Details of the editing process to be followed during the meeting will be explained early in the process.

Typically, after the (usually) first draft of the report item has been approved by the WHO group and any comments/revisions have been incorporated by the monographer and confirmed by the reviewer, the WHO rapporteur checks the revisions and, when satisfied, passes the file to the editor. The editor edits the draft report item and sends it back to the monographer to check all changes made and to answer any questions raised during the editing process.

During subsequent discussions on the report item, the editor is responsible for making all necessary revisions on-screen, until the WHO group is completely satisfied with the report item (referred to as “going to final”). At this point, the editor passes the final report item to
FAO (usually the FAO rapporteur) for its review and incorporates any changes resulting from that review.

- It is the monographer’s responsibility to keep track of any changes made to the report item that will require corresponding changes to the monograph. By the end of the meeting, all such changes to the monograph need to have been made so that the two are consistent. An electronic version of the final monograph needs to be provided to the editor before the monographer leaves the meeting on the final day.
Chapter 4: Additional considerations

The EHC monograph entitled *Principles and methods for the risk assessment of chemicals in food (EHC 240 [IPCS, 2009])* should be referred to for detailed information on hazard identification and characterization, dose–response assessment, derivation of a health-based guidance value, dietary exposure assessment and risk characterization for residues of veterinary drugs.

This chapter includes general considerations relevant to veterinary drug residues that were discussed at meetings of the Committee subsequent to publication of the above monograph. It also highlights some relevant definitions and other information from that monograph that are critical in performing risk assessments on residues of veterinary drugs.

### 4.1 Special studies on microbiological effects

The assessment of the effects of antimicrobial drug residues in food on the human intestinal microflora is routinely required by JECFA for compounds intended to have microbiological activity. However, if data are provided for other compounds, the monographer should also include an evaluation of the submitted microbiological data in these cases.

A JECFA decision-tree approach that complies with Guideline 36 of the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH, 2004, 2012) is used by the Committee to determine the need to establish a microbiological ADI for veterinary drugs (IPCS, 2009). The decision-tree approach initially seeks to determine if there may be microbiologically active veterinary drug residues entering the human colon. This is done in three steps, in which the questions are:

- **Step 1:** Are residues of the drug and/or its metabolites microbiologically active against representatives of the human intestinal flora?
- **Step 2:** Do residues enter the human colon?
- **Step 3:** Do the residues entering the human colon remain microbiologically active?

If the answer is “no” to the questions in any of the first three steps, then no microbiological ADI is necessary. However, should such residues be present, then two end-points of public health concern are to be considered: (1) disruption of the colonization barrier and (2) increase of the population(s) of resistant bacteria. At step 4 of the decision-tree process (Step 4: Is there any scientific justification to eliminate testing for either one or both end-points of concern (i.e. disruption of the colonization barrier or resistance development)?), it is possible to provide scientific justification to eliminate testing (i.e. the need for a microbiological ADI) for either one or both end-points. Step 5 (Derivation of a microbiological ADI using the VICH GL36 approach) is where a microbiological ADI would be determined.

The decision-tree approach makes use of observations in humans if such data are available. If this is the case, it is reflected in the uncertainty factor used by the Committee. However, the typical situation is that the ADI is based on in vitro MIC data. The following formula is used to derive a microbiological ADI from in vitro MIC data:

\[
\text{Upper bound of the microbiological ADI (µg/kg bw)} = \frac{\text{MIC}_{\text{calc}} \times \text{Mass of colon content}}{\text{Fraction of oral dose available to microorganisms} \times \text{Body weight} \times \text{Uncertainty factor}}
\]

where:

- The \( \text{MIC}_{\text{calc}} \) or NOAEC for colonization barrier disruption represents the lower 90% confidence limit for the mean \( \text{MIC}_{50} \) (the minimum inhibitory concentration for 50% of strains) for the most relevant and sensitive human colonic bacterial genera. An intrinsically resistant bacterial genus should not be included.

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[7](http://www.who.int/entity/foodsafety/publications/chemical-food/en/index.html)
• The mass of colon content is assumed to be 220 g based on the colon content measured from humans. It should be noted that the use of a gut volume of 220 g in the formula is a conservatively low estimate, as recent data indicate that the colon volume is, in fact, much higher than this (561 mL). The Committee will decide whether to update the value used at its next meeting.

• The fraction of an oral dose available to microorganisms is ideally based on in vivo measurements for the drug administered orally. Alternatively, if sufficient data are available, the fraction of the dose available for colonic microorganisms can be calculated as 1 minus the fraction of an oral dose excreted in urine. Human data are encouraged; in their absence, non-ruminant animal data are recommended. In the absence of data to the contrary, it should be assumed that metabolites have antimicrobial activity equal to that of the parent compound. The fraction may be lowered if the applicant provides quantitative in vitro or in vivo data to show that the drug is bound during transit through the intestine.

• The uncertainty factor is a value, usually from 1 to 10, used to account for uncertainty in the amount and relevance of the data available for review.

• The body weight of an adult human is assumed to be 60 kg.

The upper bounds of the microbiological and toxicological ADIs are compared, and, in general, the ADI is established on the basis of the lower of the two values. When a microbiological ADI is not necessary, the toxicological ADI is established as the ADI for the compound.

4.2 Guidance on establishing acute reference doses (ARfDs)

The ARfD of a chemical is an estimate of the amount of the substance in food and/or drinking-water, normally expressed on a body weight basis, that can be ingested in a period of 24 hours or less, without appreciable risk to the health of the consumer, on the basis of all the known facts at the time of the evaluation.

JMPR has established ARfDs for a number of pesticides since 1995. The suggested numerical cut-off for setting ARfDs for pesticides, recommended by JMPR, was 5 mg/kg bw; that is, if calculations indicated that an ARfD would be greater than this value, then it would not be necessary on practical grounds to establish an ARfD, as residue levels necessary to achieve this intake would be highly unlikely to occur in practice, under any conceivable use scenarios.

JECFA recognizes the importance of developing guidance for the assessment of the risk of acute exposures to residues of veterinary drugs to address situations in which it would be necessary to establish an ARfD and how this would be done. Consideration should also be given to compounds for which the ADI is based on an acute effect (e.g. pharmacological effects, antimicrobial effects).

The following principles have been agreed, which will allow a working group to develop guidance on when and how to establish ARfDs for veterinary drugs:

• The main driver for the need to consider establishing an ARfD is the toxicological profile of the compound. For a veterinary drug, high exposure can also be a consideration.

• There are currently insufficient data to determine a generic toxicological cut-off value for acute effects based on exposure considerations; hence, the decision on whether to establish an ARfD is taken after consideration, case by case, of different (realistic) acute exposure scenarios, thereby allowing practical exposure considerations. As experience is gained, it may be possible to establish such a cut-off value, as has been done for pesticides.

• An appropriate acute dietary exposure assessment method needs to be used. The principles for a suitable method were described in EHC 240 (IPCS, 2009), and details of the method were proposed in the report of the FAO/WHO workshop on dietary exposure to veterinary drugs (global estimate of acute dietary exposure, or GEADE) (FAO/WHO, 2012b).

• The Committee clarified that the theoretical maximum daily intake (TMDI) calculation is a tool used as a proxy in dietary exposure assessment, in which a standard amount of food is combined with a selected highest residue level. The standard amounts of food used in the
TMDI can be lower than the 97.5th percentile, as stated in EHC 240 (IPCS, 2009). Therefore, the TMDI is not appropriate for acute dietary exposure assessment.

- For establishing an ARfD for veterinary drugs, basic concepts as established for pesticide residues can be used. The key differences between veterinary drugs and pesticides relate to microbiological effects and to specific exposure scenarios. Regarding pharmacological effects – i.e. interaction with molecular targets (e.g. receptors) – it was noted that this is not unique to veterinary drugs and that such effects do not automatically raise an acute health concern. Such effects need to be considered for acute and chronic health effects, in the same way as for toxic effects. In practice, this may lead to the same numeric value for the ADI and ARfD.

- Misuse (e.g. off-label use) of compounds is not within the scope of these considerations, just as they are not for chronic risk assessments.

- Regarding considerations for a microbiological ARfD, the Committee recognized that an acute exposure of the gut microbiota is different from the chronic daily exposure that JECFA evaluates to establish the microbiological ADI and that the most relevant microbiological endpoint for acute exposure would most likely be disruption of the colonization barrier.

- It was noted that in extrapolating in vitro MIC\textsubscript{50} values (and other microbiological data) to an effect dose in vivo, the factors to be considered differ from those used in establishing a microbiological ADI from such data. This could result in the incorporation of a different value for the correction factor used in the formula to calculate the microbiological ADI. The specific factor to be used would be compound specific, and guidance needs to be developed on the type of information necessary to enable the Committee to estimate such a factor. Consideration will also need to be given as to what would be an appropriate default factor in the absence of compound-specific information.

- When discussing the implications for MRL recommendations, the Committee suggested to continue with MRL derivations that are compatible with chronic exposure (i.e. the ADI) and the respective withdrawal times. If an ARfD is established for the compound as well, an acute exposure assessment will then be performed based on tissue concentrations at the estimated withdrawal times, and the consequences will be described in detail. If the ARfD is exceeded, this will be reported, and possible refinements of the assessment will be described, including options such as the selection of a later time point for the recommendation of MRLs.

The draft guidance will be made available for public comments before further discussion at the next JECFA meeting dealing with veterinary drug residues in food. It was also recommended that a subgroup be established to review available information on acute exposure to residues of veterinary drugs and to identify an upper-bound exposure value with sufficient confidence that will enable, if possible, the derivation of a cut-off value for acute toxicity.

Specific detailed guidance on the establishment of ARfDs can be found in FAO/WHO (2004), Solecki et al. (2005), OECD (2010) and WHO (2015).

### 4.3 Expression of the ADI, derivation of the MRL and rounding procedures

One of the functions of JECFA is to establish health-based guidance values for residues of veterinary drugs, most often an ADI. The ADI is an output of a risk assessment of the compound, following application of the first two steps of the risk assessment paradigm: hazard identification and hazard characterization. As such, it represents a health-based guidance value, where exposure is considered to represent a negligible risk to consumers if it does not exceed the upper bound of the ADI. The ADI has a number of uses in risk assessment and risk management, including, but not limited to, helping to derive the recommended MRLs.

The MRL and the ADI are separate outputs of the risk assessment process and serve different purposes.

The ADI is established from the POD (e.g. NOAEL) from the appropriate toxicological and/or microbiological studies, using a safety factor. Given that there are assumptions and uncertainties in establishing the ADI, such as the use of safety factors, the use of a range of doses in toxicological studies and normal biological variation, it is more meaningful to express the ADI to only one significant figure to avoid any inference of inappropriate precision. If an ADI is calculated from a POD that has
more than one significant figure, the ADI would therefore be rounded to one significant figure, consistent with accepted rounding procedures.

The general rounding rule for mid-way values (x.5) is to round up, in line with common convention (e.g. Standards Australia International, 2003). Examples for rounding to one significant figure are as follows: 1.25 becomes 1, 0.73 becomes 0.7 and 1.5 becomes 2.

JECFA has confirmed that the rounding practices used in expressing the ADI are scientifically and mathematically sound. In addition, as the ADI is not used directly in the derivation of the MRL, the JECFA rounding practices have no direct consequence on the numerical value of the MRL.

4.4 Overall NOAEL

JECFA generally uses the lowest NOAEL in the most sensitive species, usually among mice, rats and dogs, to establish the ADI; however, there might be situations where there is more than one study in which the same end-points have been addressed. In such situations, the dose spacing may be different, resulting in different NOAELs and LOAELs. In such circumstances, it might be appropriate to consider the studies together. When they are comparable, including consideration of study design, duration, end-points addressed, and species and strain of animal, an “overall NOAEL” is identified, which is the highest NOAEL in the available studies, provided that there is a reasonable margin (≥ 2) over the lowest LOAEL (which becomes to the “overall LOAEL”) and that due consideration is given to the shape of the dose–response curve.

4.5 Assessment of short-term (90-day and 12-month) studies in dogs

Following analysis of a number of databases comprising information from several hundred compounds, including many pesticides, many authorities (including the USEPA, European Commission, JMPR) concluded that the nature and potency of effects observed after oral administration to dogs for 90 days rarely showed any change after a further 9 months of administration; in other words, the effects and the NOAELs at 12 months were the same as at 90 days. As a consequence, it was recommended that there was need for only a 90-day study in dogs, and this has since been reflected in the OECD test guideline for short-term studies in dogs.

JMPR noted that in light of this, it would be possible to consider most 90-day and 12-month studies in dogs to be short-term repeated-dose studies providing the same information. Hence, following the same considerations as for two studies of the same duration, it would be possible to identify an overall NOAEL (and LOAEL) for the studies (see section 4.4 above). It was agreed that JECFA would adopt the same practice.

Therefore, where both a 13-week and longer-term (usually 1-year) dog studies are provided, an overall NOAEL and LOAEL should be identified, unless this is not feasible (e.g. NOAELs are not consistent).

4.6 WHO list of critically important antimicrobials

Over the past years, concern has increased over the use of antimicrobials in food animals and whether certain uses can create an important source of antimicrobial-resistant bacteria that can spread to humans through the food supply. WHO has developed and applied criteria to rank antimicrobials according to their relative importance in human medicine and has established and regularly updates the list of “Critically Important Antimicrobials in Human Medicine”.  

JECFA agreed to make reference to this list and note in the Explanation section (preferably at the end of this section) if the compound is listed.

Example: The World Health Organization has categorized [compound] as a critically important antimicrobial in human medicine.

4.7 Use of a margin of exposure approach

JECFA will generally establish health-based guidance values for veterinary drugs. However, when this is not possible or not appropriate, the Committee might report a margin of exposure (MOE). There are primarily two situations when this might be done: (1) when the nature of the end-point is such that derivation of a health-based guidance value is not appropriate, and (2) when there are deficiencies in the database such that it is not possible to derive a health-based guidance value with confidence.

The most common example of the first situation is for compounds that JECFA considers to be both genotoxic and carcinogenic, for which JECFA typically considers it inappropriate to establish an ADI. The same general rule also applies to metabolites of the parent compound, if present as residues, whether or not the parent compound is itself genotoxic and carcinogenic. For example, the Committee concluded that gentian violet should be considered a carcinogen acting by a genotoxic mode of action in view of the carcinogenicity of gentian violet in the mouse and rat and evidence showing genotoxicity in a number of tests; therefore, the Committee could not establish an ADI for this compound (FAO/WHO, 2014). Similarly, the Committee concluded that the use of malachite green in food-producing animals could not be supported because its major metabolite, leucomalachite green, induced hepatocellular adenomas and carcinomas in female mice, and it could not be ruled out that this was occurring by a genotoxic mode of action (FAO/WHO, 2009).

For compounds that are genotoxic and carcinogenic, the Committee would not normally establish an ADI or recommend MRLs if the substances give rise to residues detectable in human food by analytical methods with an appropriate limit of detection. Notwithstanding, there may be some situations where JECFA is requested to provide advice following exposure to such compounds in the diet. Examples would be illegal use, former use leading to residues as contaminants, and impurities or metabolites in drugs that are not themselves genotoxic and carcinogenic.

In this situation, JECFA has adopted an MOE approach in which the estimated human exposure is compared with the lowest POD for the effect of concern in order to provide advice to risk managers. The MOE is derived by taking the ratio of the POD to the most relevant, sensitive end-point to an estimate of exposure by a high consumer. For compounds that are genotoxic and carcinogenic, the preferred POD is the BMDL[10], when using data from carcinogenicity studies in animals. Where a BMDL cannot be determined, owing to the nature of the available data, the T[25] (the chronic daily dose in mg/kg bw that will give 25% of the animals tumours at a specific tissue site, after correction for spontaneous incidence, within the standard lifespan of that species) may provide an alternative POD. However, the suitability of the T[25] for a given data set needs to be considered on a case-by-case basis.

The second situation in which the MOE approach might be used arises when the toxicological database is incomplete. In such cases, it should be noted that the most sensitive end-point might not have been evaluated, and hence it might not be possible to establish a health-based guidance value with confidence. Further, it might not be possible to derive an MRL for the substance, in which case exposure will need to be estimated from available residue data, using the same principles as used for deriving a MRL, to the extent possible.

In general, the interpretation of an MOE is based on considerations similar to those used in establishing a health-based guidance value. Hence, when based on data from experimental animals, as a default, an MOE of at least 100 (10-fold for each of interspecies and intraspecies variability) would be considered as an indication for low health concern for effects with an apparent threshold. For compounds with carcinogenic and genotoxic properties, JECFA (in the case of additives and contaminants) has indicated a low health concern for compounds with an MOE above 10 000. In interpreting the MOE, consideration needs to be given to all relevant factors, including the conservatism of assumptions, the completeness of the database (have all potentially relevant end-points been assessed?), whether the response might reasonably be considered to exhibit a biological threshold and whether residues arise through permitted use, inadvertently or unavoidably. These need to be clearly described in the report.

It is important to note that an MOE is not an absolute value but rather a relative comparison, intended to provide some indication to risk managers as to the level of concern and help in assessing the need for and urgency of further action. For substances that are both genotoxic and carcinogenic, this approach provides advice to inform risk managers of how close human exposures are to those anticipated to produce a measurable effect in laboratory animals or humans. In addition, MOEs for different substances can be compared to assist risk managers in prioritizing risk management actions.
MOEs should be rounded to at most two significant figures to avoid spurious precision.

4.8 Benchmark dose

Where considered appropriate, the Committee may choose to use the benchmark dose (BMD) approach (e.g. using the United States Environmental Protection Agency’s [USEPA] Benchmark Dose Software [BMDS]) for modelling dose–response relationships, as such modelling will result in a more reliable estimation of the POD for use in risk assessment. However, in many instances, the NOAEL will provide an adequate POD for risk assessment, and in some cases, the data are not amenable to BMD modelling. The decision to employ a BMD approach is therefore made on a case-by-case basis by the monographer reviewing the dossier in consultation with the reviewer assigned to the compound.

The principle is described in the report of the forty-fourth JECFA meeting (FAO/WHO, 1995), and several examples illustrating the use of the BMD approach for dose–response modelling can be found in the report of the seventy-eighth JECFA (FAO/WHO, 2014).

4.9 Dietary exposure assessment for veterinary drug residues

Dietary exposure assessment plays an essential part in quantifying risk and is central to the work of JECFA. There has been an ongoing need to improve the approaches used to estimate dietary exposure to veterinary drug residues in foods. An expert meeting on dietary exposure assessment methodologies for residues of veterinary drugs, held in November 2011 (FAO/WHO, 2012b), proposed new methods for acute and chronic dietary exposure assessment for veterinary drug residues, taking the key findings, concerns and recommendations of stakeholders into consideration. Subsequently, it was recommended that the new approaches should be piloted at the seventy-eighth meeting of JECFA. The purpose of the pilot study was to explore the new calculations for dietary exposure assessment (for four veterinary drug residues), compare them with estimates calculated using the model diet approach, identify the practical impact of using the new methods and make recommendations for dietary exposure assessment at future meetings.

The current model diet used for veterinary drug residues (the so-called food basket model) is intended to cover chronic high consumers of animal products. The model assumes that the food consumption applies to an adult with a body weight of 60 kg and is intended to also cover the consumption of all processed foods with these foods as ingredients. All muscle tissues are treated as equivalent, so meat and fish consumed are considered as equivalent in the calculations.

For estimating chronic dietary exposures to veterinary drug residues, JECFA uses the median of the residue depletion to derive the estimated daily intake (EDI). The contribution to the EDI from consumption of individual tissues is calculated by multiplying the amount of tissue in the model diet by the median concentration of marker residue corresponding to the MRL. The EDI itself is the sum of the individual intakes resulting from all tissues. Where a median residue cannot be derived, the MRL may be substituted for the median residue to calculate the theoretical maximum daily intake (TMDI).

The two new methods for estimating dietary exposure are the global estimate of acute dietary exposure (GEADE) and the global estimate of chronic dietary exposure (GECD). Both methods differ from the EDI by being based on individual food consumption from surveys rather than on a standard basket and by using more realistic global consumption amounts as inputs into the calculations. Instead of the set amounts of food in the model diet, more detailed food consumption data are used where available. For example, muscle tissue is differentiated by species, and finfish are considered separately from molluscs and crustaceans. This allows the estimation of specific dietary exposures for additional population groups (children aged 12 months and older and infants younger than 12 months). Consumption data used can be expressed per person, to be compared with the current approach, or per kilogram body weight, based on values reported in food consumption surveys.

It should be noted that consumption amounts for infants are not reported for some categories (e.g. mammalian fat, poultry fat and skin) and therefore are not included in estimates. Other categories were not reported separately as consumed according to the surveys used to derive consumption amounts. In such cases, the broader categories have been used, with the highest residue concentration used as the input. For example, “mammalian kidney” consumption is not reported for
infants: therefore, the residue found in kidney would be assigned to “All mammalian offal”, which is the best available match for kidney consumption in this population.

The current food consumption model does not adequately estimate acute dietary exposure even when the standard food basket is combined with high residue levels. An adequate model should be based on the highest probable consumption from a single commodity on a single day or over a single meal (and is intended to be combined with a high residue level and used for comparison with ARfD values in a risk assessment process). The GEADE is an explicit estimate of acute dietary exposure, combining consumption at the 97.5th percentile with the 95th percentile residue concentration. Estimates can be derived specifically for children as well as for the general population, following the principle that dietary exposure assessments should cover the whole population, including children.

The GECDE uses median residues combined with two different types of consumption data to estimate chronic dietary exposure. Firstly, the highest exposure at the 97.5th percentile of consumption is selected from the 97.5th percentiles of consumption for all the foods relevant to exposure. This value is derived from chronic consumers of the food; that is, the percentile consumption is calculated from consumers of the food only and is different from the 97.5th percentile of consumption used in acute exposure, which reflects a single eating occasion (acute). Secondly, the mean dietary exposures from all the other relevant foods are then added to estimate total exposure. The mean dietary exposure is derived from the total population; in other words, non-consumers of the food are included in the mean calculation. In addition to the general population and children, dietary exposure of infants can also be estimated.

Results of the pilot study are discussed in the seventy-eighth report of JECFA (FAO/WHO, 2014). It was recommended that the new approach for chronic dietary exposure (GECDE) should continue to be used in parallel with the model diet approach at future meetings of the Committee until more experience has been obtained in the interpretation of the results with the new approach. The model for acute dietary exposure (GEADE) should be used when an ARfD is established by JECFA. A number of areas were also identified that should be investigated to further improve dietary exposure methodology for residues of veterinary drugs.

Two important issues concerned with the methodologies applied by JECFA and JMPR to estimate chronic dietary exposures have been identified. The first issue is how to estimate chronic exposure to veterinary drug residues where it is known that the compound of interest is also used as a pesticide. Several alternatives were proposed, including the most comprehensive approach of developing a specific chronic exposure model that would fit both JMPR and JECFA risk assessment purposes. The second issue is the potential need for dietary exposure assessment methods that take into account short-term toxicity after less-than-lifetime exposures. An expert working group was established by FAO and WHO to address these two issues and should report back to JECFA and JMPR.

4.10 Avermectins and the CF-1 mouse

The Committee and JMPR have evaluated several members of the group of compounds known as avermectins. A consistent observation was that the CF-1 mouse was particularly sensitive to the toxicity of these compounds. In the absence of information to the contrary, the NOAEL in the CF-1 mouse was therefore regarded as the critical NOAEL and served as the basis for establishing the respective ADI.

Advances in understanding of the membrane transport protein P-glycoprotein (ABCB1) in the mid-1990s led to the realization that avermectins are substrates for this transporter and that their potency in the CF-1 mouse is a consequence of a natural mutation leading to lack of expression of this protein. Two other examples of genetic absence of P-glycoprotein are known, in subpopulations of Collie dogs and Murray red cattle; no other strain or species is known in which this occurs. Studies in humans have established that P-glycoprotein is expressed in all individuals and is expressed at near adult levels in the newborn. Although polymorphisms of P-glycoprotein are known in humans, their impact on the transport activity of the protein is relatively modest.

Such knowledge led JECFA and JMPR in their recent evaluations of avermectins to discount effects observed in the CF-1 mouse in identifying critical effects for the establishment of ADIs. P-glycoprotein expression in rats is immature compared with that in humans, so that developmental effects of avermectins in rats and effects in young pups can, in general, be discounted as not being relevant to humans. However, effects in older pups should be assessed on a case-by-case basis.
4.11 Injection site residues

JECFA has noted on occasion that residues at injection sites may exceed the recommended MRL for the tissue or tissues concerned at practical withdrawal times. To assess the safety implications of residues at the injection site, JECFA requires information on concentrations of residues observed in injection sites sampled under standardized conditions. The Committee has accepted a sampling procedure required by both the European Medicines Agency (EMEA) and the United States Food and Drug Administration. It was noted that the EMEA has recently modified its sampling procedure, which now requires, in addition to a core sample of 500 g, a second sample of tissue surrounding the core sample in order to confirm the quality and correctness of the original sampling (EMEA, 2004).

JECFA assesses the safety of injection site residues by comparison with an ARfD (e.g. carazolol in injection sites, evaluated at the fifty-second meeting of JECFA; FAO/WHO, 2000). This assessment should be done by combining the residues at the injection site with the high consumption figures from the GEADE model. In contrast, JECFA does not include residues that persist at or near the injection site in assessing the contribution of drug residues in edible tissues to the estimated chronic daily exposure.

4.12 Guidance on the interpretation of hepatocellular hypertrophy

Annex D of the JMPR guidance for monographers and reviewers (WHO, 2015) focuses on the histological observation of hepatocellular hypertrophy, a change to the liver that is commonly observed in toxicological studies, particularly in rodents. The purpose of this annex is to provide general guidance for determining whether the observation of hepatocellular hypertrophy in different laboratory species is indicative of an adaptive or an adverse event, so that the most appropriate reference dose can be identified for the establishment of health-based guidance values.

Rather than repeating this annex here, the reader is referred to the JMPR guidance document (http://www.who.int/foodsafety/publications/jmpr_guidance_document_1.pdf?ua=1) for more detailed information.

The following general principles were developed by JMPR to be followed in the final assessment of liver hypertrophy:

- In the absence of histopathological damage and relevant clinical chemistry changes at the dose that induces only hepatocellular hypertrophy and/or liver size/weight changes, hypertrophy should not be identified as an adverse effect or used for establishing health-based guidance values. This dose should be identified as a lowest-observed-effect level (LOEL).
- If hepatotoxicity, as characterized by toxicologically significant changes in histopathology and/or clinical chemistry, occurs at doses higher than those causing liver hypertrophy, then the LOAEL for the study should be the dose that elicits hepatotoxicity (or some other relevant toxicity found in the study).
- If there is insufficient information to determine whether the observed liver hypertrophy is an adaptive or an adverse response, then the default is to assume that the effect is adverse.
- If other organ responses are observed that may be the secondary consequence of enhanced hepatic metabolism (e.g. increased hepatic clearance of thyroid hormones), these effects should be evaluated for their relevance to the establishment of health-based guidance values.
- The established health-based guidance value (e.g. ADI) should not be greater than the LOEL for the induction of xenobiotic metabolizing enzymes. If potency for induction were such that this would occur, the POD for induction should be considered in establishing the respective health-based guidance value.

The observation of hyperplasia or neoplasia would usually trigger consideration of the mode of action for this effect. Liver hypertrophy and a range of other morphological changes can result from chemically mediated effects on different nuclear receptors, but not all of these mechanisms are necessarily relevant to humans. The International Programme on Chemical Safety framework for analysing the mode of action of an agent in causing hepatic effects in animals and its relevance for humans should be followed (Boobis et al., 2006).
Chapter 5. A risk-based decision-tree approach for the safety evaluation of residues of veterinary drugs

The JECFA Secretariat convened a working group to develop a general decision-tree for the evaluation of veterinary drugs, which would identify different options for hazard identification, hazard characterization and exposure assessment, and a draft was presented at the seventieth meeting of JECFA. Following discussion, the draft was revised, and the paper, entitled “A risk-based decision-tree approach for the safety evaluation of residues of veterinary drugs”, was submitted to the Eighteenth Session of CCRVDF as “work-in-progress”. CCRVDF agreed with the proposed general principles, supported further work on this matter and recommended that the JECFA Secretariat also establish an electronic working group to elaborate principles to establish ARfDs for residues of veterinary drugs. At its seventy-fifth meeting, JECFA further considered the proposed decision-tree approach for the safety evaluation of residues of veterinary drugs and agreed to the following:

- Preliminary risk assessment, as envisaged in the decision-tree, would be most readily accomplished by Member States when considering suggesting compounds for evaluation by JECFA. The Committee recommended that an electronic working group be established to develop guidance on what would comprise a preliminary risk assessment, taking into account the risk analysis principles applied by CCRVDF.

- A number of issues would need to be addressed in applying the threshold of toxicological concern (TTC) approach to residues of veterinary drugs. In particular, there were very few pharmacological (receptor-mediated) effects in the database used to derive the existing TTC values, and some such effects can occur at particularly low doses. The Committee recommended that an electronic working group be established to perform a feasibility exercise on the application of the TTC approach to residues of veterinary drugs and, if appropriate, to make specific recommendations for developing such an application.

- The Committee confirmed the importance of developing guidance for the acute risk assessment of residues of veterinary drugs and recommended that an electronic working group be established to develop guidance for establishing ARfDs for residues of veterinary drugs, addressing situations in which it would be necessary to establish an ARID and how this would be done. Consideration should also be given to compounds for which the ADI is based on an acute effect (e.g. pharmacological effects, antimicrobial effects). The working group should include an expert from JMPR who is experienced in the establishment of ARfDs (see section 4.2 above).

The final guidance on the application of the decision-tree will be included in future updates of this guidance document.

The paper entitled “A risk-based decision-tree approach for the safety evaluation of residues of veterinary drugs" is available on the web at: http://www.who.int/entity/foodsafety/chem/jecfa/decision_tree_mar_2009_final_for_web.pdf?ua=1


VICH (2012). Studies to evaluate the safety of residues of veterinary drugs in human food: general approach to establish a microbiological ADI. Brussels: International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH GL36 (R)).

Annex 1: Template for monograph

A sample table of contents for the toxicological monographs is given below. Not all headings will be applicable in all cases; “No information was available” can be inserted under main headings (usually up to third level), or minor headings that are not applicable can be deleted.

It should be noted that the design of the monographs was changed in 2015, which has resulted in several formatting changes. Text is 12 pt Times New Roman, headings are 14/12/10/9 pt Arial, paper size A4, 1 inch margins, single spaced, first line of first paragraph under each heading is flush left, first line of each subsequent paragraph indented 0.5 inch, no spacing between paragraphs, paragraphs are fully justified, extra line space is added between different study descriptions, line numbering is required for the draft monograph.

Veterinary drug name (addendum, if applicable)

First draft prepared by
Author 1,1 Author 22 … and Author x x

1 Affiliation of author 1
2 Affiliation of author 2
...
 x Affiliation of author x

(include names and affiliations of all experts who contributed to the draft, including WHO and FAO authors and reviewers; main WHO author is given first, followed by rest of contributors in alphabetical order)

1. Explanation
2. Biological data
   2.1 Biochemical aspects
      2.1.1 Absorption, distribution and excretion
      2.1.2 Biotransformation
      2.1.3 Effects on enzymes and other biochemical parameters
   2.2 Toxicological studies
      2.2.1 Acute toxicity
         (a) Lethal doses
         (b) Dermal irritation
         (d) Ocular irritation
         (e) Dermal sensitization
      2.2.2 Short-term studies of toxicity
         (a) Mice
         (b) Rats
         (c) Hamsters
         (d) Rabbits
         (e) Dogs
         (f) Pigs
         (g) Monkeys
      2.2.3 Long-term studies of toxicity and carcinogenicity
         (a) Mice
         (b) Rats
      2.2.4 Genotoxicity
      2.2.5 Reproductive and developmental toxicity
(a) Multigeneration reproductive toxicity
(b) Developmental toxicity

2.2.6 Special studies *(below are examples only; note alphabetical order)*
(a) Cardiovascular effects
(b) Immune responses
(c) Metabolites
(d) Neurobehavioural effects
(e) Ocular toxicity
(f) Thyroid function

2.3 Microbiological effects
2.4 Observations in humans

3. Comments
   3.1 Biochemical data
   3.2 Toxicological data
   3.3 Microbiological data

4. Evaluation

5. References
### Table 1
**Relative distribution of metabolites in male rat urine (0–24 hours post-dosing) administered teflubenzuron by gavage**

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>% of total metabolites</th>
<th>Metabolite designation</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,6-Difluorobenzoic acid</td>
<td>81.4 ± 3.8</td>
<td>V</td>
</tr>
<tr>
<td>2,6-Difluorobenzamide</td>
<td>2.0 ± 0.3</td>
<td>VI</td>
</tr>
<tr>
<td>(3,5-Dichloro-2-fluoro-4-phenylglucuronidephenyl)urea</td>
<td>5.2 ± 2.4</td>
<td>VIII</td>
</tr>
<tr>
<td>2,6-Difluorobenzoylglycine</td>
<td>4.0 ± 0.5</td>
<td>IX</td>
</tr>
<tr>
<td>(3,5-Dichloro-2-fluoro-4-phenylsulfatephenyl)urea</td>
<td>0.3 ± 0.6</td>
<td>X</td>
</tr>
<tr>
<td>4-Amino-2,6-dichloro-3-fluorophenylsulfate</td>
<td>1.2 ± 0.4</td>
<td>XI</td>
</tr>
<tr>
<td>4-Acetamido-2,6-dichloro-3-fluorophenylsulfate</td>
<td>0.6 ± 0.5</td>
<td>XII</td>
</tr>
<tr>
<td>2-Amino-3,5-difluoro-4,6-dichlorophenylsulfate</td>
<td>2.8 ± 0.5</td>
<td>XIII</td>
</tr>
<tr>
<td>2-Amino-3,5-difluoro-4,6-dichlorophenylglucuronide</td>
<td>1.2 ± 0.3</td>
<td>XIV</td>
</tr>
<tr>
<td>Unidentified</td>
<td>1.3 ± 0.7</td>
<td>–</td>
</tr>
</tbody>
</table>

*See Fig. 2.*  
**Source:** After Koerts et al. (1997)

### Table 2
**Comparison of pharmacokinetics data following administration of a single oral dose of ivermectin to dogs**

<table>
<thead>
<tr>
<th>Breed (number)</th>
<th>Dose (µg/kg bw)</th>
<th>t½ (days)</th>
<th>T&lt;sub&gt;max&lt;/sub&gt; (day)</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (ng/mL)</th>
<th>Dose-normalized C&lt;sub&gt;max&lt;/sub&gt; (ng/mL)</th>
<th>AUC (ng·d/mL)</th>
<th>Dose-normalized AUC (ng·d/mL)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beagle (9)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>81.5</td>
<td>4.4</td>
<td>0.22</td>
<td>24.0</td>
<td>0.3</td>
<td>38.9</td>
<td>11.5</td>
<td>Dunn et al. (2011)</td>
</tr>
<tr>
<td>Beagle (16)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100</td>
<td>ND</td>
<td>0.18</td>
<td>44.3</td>
<td>0.4</td>
<td>43</td>
<td>6.7</td>
<td>Daurio et al. (1992)</td>
</tr>
<tr>
<td>Cross-breed (5)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>200</td>
<td>3.3</td>
<td>0.23</td>
<td>116.8</td>
<td>0.6</td>
<td>237</td>
<td>28</td>
<td>Gokbulut et al. (2006)</td>
</tr>
<tr>
<td>Beagle (8)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>250</td>
<td>3.3</td>
<td>0.17</td>
<td>132.6</td>
<td>0.5</td>
<td>233</td>
<td>22.4</td>
<td>Al-Azzam et al. (2007)</td>
</tr>
<tr>
<td>Not defined (12)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>300</td>
<td>1.8</td>
<td>0.30</td>
<td>9.64</td>
<td>0.03</td>
<td>27.8</td>
<td>2.2</td>
<td>Gong et al. (2010)</td>
</tr>
<tr>
<td>Beagle (10)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>300</td>
<td>2.07</td>
<td>0.29</td>
<td>92.70</td>
<td>0.31</td>
<td>141.96</td>
<td>11.4</td>
<td>Walther, Allan &amp; Roepke (2015)</td>
</tr>
</tbody>
</table>

<sup>a</sup> See Fig. 2.  
**Reference:** After Koerts et al. (1997)
AUC: area under the plasma concentration–time curve; bw: body weight; $C_{\text{max}}$: peak plasma concentration; ND: no data; $t_\text{½}$: elimination half-life; $T_{\text{max}}$: time to reach $C_{\text{max}}$

a. Sex not defined. Body weight not defined. Fasting status not provided.
b. Sixteen females. Body weight not defined. Fasting status not provided.
c. Five females (15–30 kg). Not fasted.
d. Four of each sex (7.9–16.3 kg) inoculated with *Brugia pahangi*. Fasting status not provided.
e. Six of each sex (mean body weight ± SD: 9.80 ± 0.48 kg). Fasted.
f. Five of each sex (mean body weight ± SD: 13.1 ± 1.2 kg). Fasting status not provided.

Table 3
Acute toxicity studies on sisapronil

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of each sex per group</th>
<th>Route</th>
<th>Dose (mg/kg bw)</th>
<th>$LD_{50}$ (mg/kg bw)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>9 F</td>
<td>Oral</td>
<td>175, 550, 2 000</td>
<td>552</td>
<td>Beerens-Heijnen (2011a)</td>
</tr>
<tr>
<td>Rat</td>
<td>2 M</td>
<td>Oral</td>
<td>100, 250, 500, 1 000, 1 500</td>
<td>Not estimated</td>
<td>Gagnon (2012a)</td>
</tr>
<tr>
<td>Rat</td>
<td>3 M</td>
<td>Oral</td>
<td>10</td>
<td>Not applicable</td>
<td>Gagnon (2012b)</td>
</tr>
<tr>
<td>Rat</td>
<td>20 M</td>
<td>Oral</td>
<td>0, 100, 500, 1 000</td>
<td>Not estimated</td>
<td>Ryan (2011)</td>
</tr>
<tr>
<td>Mouse</td>
<td>4, sex not specified</td>
<td>Intravenous</td>
<td>0.3, 1, 3</td>
<td>&gt; 3</td>
<td>Mill (2003)</td>
</tr>
</tbody>
</table>

bw: body weight; F: females; $LD_{50}$: median lethal dose; M: males

Table 4
Findings in rats given diflubenzuron in the diet for up to 104 weeks

<table>
<thead>
<tr>
<th>Finding</th>
<th>Sex</th>
<th>0 mg/kg feed</th>
<th>160 mg/kg feed</th>
<th>620 mg/kg feed</th>
<th>2 500 mg/kg feed</th>
<th>10 000 mg/kg feed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocyte count ($10^6$/mm$^3$)</td>
<td>Male</td>
<td>8.7</td>
<td>8.3</td>
<td>7.6*</td>
<td>8.0</td>
<td>7.4*</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>8.1</td>
<td>7.8</td>
<td>7.6</td>
<td>7.1*</td>
<td>6.5*</td>
</tr>
<tr>
<td>Haemoglobin (g/dL)</td>
<td>Males</td>
<td>17</td>
<td>16</td>
<td>15*</td>
<td>16</td>
<td>15*</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>15</td>
<td>15</td>
<td>14</td>
<td>14*</td>
<td>13*</td>
</tr>
<tr>
<td>Erythrocyte volume fraction (%)</td>
<td>Male</td>
<td>48</td>
<td>48</td>
<td>44</td>
<td>46</td>
<td>43*</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>46</td>
<td>46</td>
<td>45</td>
<td>43</td>
<td>41*</td>
</tr>
<tr>
<td>Reticulocyte count (%)</td>
<td>Male</td>
<td>0.9</td>
<td>0.8</td>
<td>1.6</td>
<td>1.7*</td>
<td>2.1*</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>1.0</td>
<td>1.1</td>
<td>1.8</td>
<td>3.1*</td>
<td>5.0*</td>
</tr>
<tr>
<td>Methaemoglobin (%)</td>
<td>Male</td>
<td>0.2</td>
<td>2.0*</td>
<td>1.5</td>
<td>2.4*</td>
<td>2.5*</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>0.6</td>
<td>0.9</td>
<td>1.4</td>
<td>1.9*</td>
<td>2.2*</td>
</tr>
<tr>
<td>Sulfhaemoglobin (%)</td>
<td>Male</td>
<td>0.1</td>
<td>1.3*</td>
<td>0.9*</td>
<td>0.7</td>
<td>1.1*</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>0.1</td>
<td>0.2</td>
<td>1.0*</td>
<td>0.1</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Week 104
<table>
<thead>
<tr>
<th>Finding</th>
<th>Sex</th>
<th>0 mg/kg feed</th>
<th>160 mg/kg feed</th>
<th>620 mg/kg feed</th>
<th>2500 mg/kg feed</th>
<th>10000 mg/kg feed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methaemoglobin (%)</td>
<td>Male</td>
<td>0.8</td>
<td>1.1</td>
<td>1.3</td>
<td>1.8*</td>
<td>1.8*</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>0.7</td>
<td>1.3</td>
<td>1.6*</td>
<td>2.1*</td>
<td>2.3*</td>
</tr>
<tr>
<td>Sulfhaemoglobin (%)</td>
<td>Male</td>
<td>0.2</td>
<td>0.2</td>
<td>0.6</td>
<td>0.8</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>0.2</td>
<td>0.3</td>
<td>0.6</td>
<td>1.1*</td>
<td>1.1*</td>
</tr>
<tr>
<td>Myeloid:erythroid cell ratio</td>
<td>Male</td>
<td>2.5</td>
<td>1.6</td>
<td>1.8</td>
<td>1.5*</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>1.9</td>
<td>1.1*</td>
<td>1.3</td>
<td>1.3</td>
<td>1.1*</td>
</tr>
<tr>
<td>Absolute spleen weight (g)</td>
<td>Male</td>
<td>1.0</td>
<td>1.0</td>
<td>1.3</td>
<td>1.4*</td>
<td>1.5*</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>0.7</td>
<td>0.9</td>
<td>0.9</td>
<td>1.1*</td>
<td>1.2*</td>
</tr>
<tr>
<td>Sternum, marrow hyperplasia (%)</td>
<td>Male</td>
<td>16</td>
<td>10</td>
<td>8</td>
<td>60*</td>
<td>72*</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>4</td>
<td>6</td>
<td>2</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Sternum, erythroid hyperplasia (%)</td>
<td>Male</td>
<td>4</td>
<td>6</td>
<td>26*</td>
<td>38*</td>
<td>30*</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>1</td>
<td>2</td>
<td>12*</td>
<td>22*</td>
<td>26*</td>
</tr>
<tr>
<td>Liver, pigmented macrophages (%)</td>
<td>Male</td>
<td>22</td>
<td>20</td>
<td>50*</td>
<td>82*</td>
<td>72*</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>26</td>
<td>38</td>
<td>74*</td>
<td>80*</td>
<td>86*</td>
</tr>
<tr>
<td>Spleen, pigmented macrophages (%)</td>
<td>Male</td>
<td>66</td>
<td>84</td>
<td>86</td>
<td>88</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>80</td>
<td>90</td>
<td>100</td>
<td>94</td>
<td>100</td>
</tr>
<tr>
<td>Stomach, acanthosis or hyperkeratosis (%)</td>
<td>Male</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>11*</td>
<td>24*</td>
</tr>
<tr>
<td>Stomach, gastritis (%)</td>
<td>Male</td>
<td>0/49 (0%)</td>
<td>0/50 (0%)</td>
<td>0/50 (0%)</td>
<td>2/50 (4%)</td>
<td>2/50 (4%)</td>
</tr>
<tr>
<td>Stomach, acanthosis or hyperkeratosis (%)</td>
<td>Female</td>
<td>0/49 (0%)</td>
<td>1/50 (2%)</td>
<td>2/50 (4%)</td>
<td>17/50 (34%)**</td>
<td>19/50 (38%)**</td>
</tr>
<tr>
<td>Haemangiosarcoma</td>
<td>Male</td>
<td>0/49 (0%)</td>
<td>0/50 (0%)</td>
<td>0/50 (0%)</td>
<td>4/50 (8%)</td>
<td></td>
</tr>
<tr>
<td>Fibrosarcoma, osteosarcoma or</td>
<td>Female</td>
<td>0/49 (0%)</td>
<td>1/50 (2%)</td>
<td>3/50 (6%)</td>
<td>38/50 (76%)**</td>
<td></td>
</tr>
<tr>
<td>haemangiosarcoma, combined</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*: P < 0.05  
Source: Burdock et al. (1984)

Table 5  
Incidences of findings in the spleen and adrenal gland in rats treated with PCA

<table>
<thead>
<tr>
<th>Findings</th>
<th>Control</th>
<th>Low dose</th>
<th>Mid dose</th>
<th>High dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spleen, males</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibrosis</td>
<td>3/49 (6%)</td>
<td>11/50 (22%)</td>
<td>12/40 (24%)</td>
<td>41/50 (82%)</td>
</tr>
<tr>
<td>Fibroma</td>
<td>0/49 (0%)</td>
<td>0/50 (0%)</td>
<td>0/50 (0%)</td>
<td>2/50 (4%)</td>
</tr>
<tr>
<td>Fibrosarcoma</td>
<td>0/49 (0%)</td>
<td>1/50 (2%)</td>
<td>2/50 (4%)</td>
<td>17/50 (34%)**</td>
</tr>
<tr>
<td>Osteosarcoma</td>
<td>0/49 (0%)</td>
<td>0/50 (0%)</td>
<td>1/50 (2%)</td>
<td>19/50 (38%)**</td>
</tr>
<tr>
<td>Haemangiosarcoma</td>
<td>0/49 (0%)</td>
<td>0/50 (0%)</td>
<td>0/50 (0%)</td>
<td>4/50 (8%)</td>
</tr>
<tr>
<td>Fibrosarcoma, osteosarcoma or haemangiosarcoma, combined</td>
<td>0/49 (0%)</td>
<td>1/50 (2%)</td>
<td>3/50 (6%)</td>
<td>38/50 (76%)**</td>
</tr>
<tr>
<td>Spleen, females</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibrosis</td>
<td>1/50 (2%)</td>
<td>2/50 (4%)</td>
<td>3/50 (6%)</td>
<td>42/50 (84%)</td>
</tr>
<tr>
<td>Fibrosarcoma</td>
<td>0/50 (0%)</td>
<td>0/50 (0%)</td>
<td>1/50 (2%)</td>
<td>0/50 (0%)</td>
</tr>
<tr>
<td>Osteosarcoma</td>
<td>0/50 (0%)</td>
<td>0/50 (0%)</td>
<td>0/50 (0%)</td>
<td>1/50 (2%)</td>
</tr>
</tbody>
</table>
### Findings

<table>
<thead>
<tr>
<th>Adrenal gland, males</th>
<th>Control</th>
<th>Low dose</th>
<th>Mid dose</th>
<th>High dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medullary hyperplasia</td>
<td>16/49 (31%)</td>
<td>21/48 (44%)</td>
<td>15/48 (31%)</td>
<td>17/49 (35%)</td>
</tr>
<tr>
<td>Phaeochromocytoma</td>
<td>13/49 (27%)</td>
<td>14/48 (29%)</td>
<td>14/48 (29%)</td>
<td>25/49 (51%)*</td>
</tr>
<tr>
<td>Malignant phaeochromocytoma</td>
<td>1/49 (2%)</td>
<td>0/48 (0%)</td>
<td>1/48 (2%)</td>
<td>1/49 (2%)</td>
</tr>
<tr>
<td>Phaeochromocytoma or malignant phaeochromocytoma, combined</td>
<td>13/49 (27%)</td>
<td>14/48 (29%)</td>
<td>15/48 (31%)</td>
<td>26/49 (53%)*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Adrenal gland, females</th>
<th>Control</th>
<th>Low dose</th>
<th>Mid dose</th>
<th>High dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medullary hyperplasia</td>
<td>4/50 (8%)</td>
<td>4/50 (8%)</td>
<td>7/50 (14%)</td>
<td>24/50 (48%)</td>
</tr>
<tr>
<td>Phaeochromocytoma</td>
<td>2/50 (4%)</td>
<td>3/50 (6%)</td>
<td>1/50 (2%)</td>
<td>6/50 (12%)</td>
</tr>
</tbody>
</table>

*: \( P < 0.05 \); **: \( P < 0.01 \) (logistic regression test)

Source: USNTP (1989)

### Table 6

**Results of genotoxicity assays on teflubenzuron**

<table>
<thead>
<tr>
<th>Test system</th>
<th>Test object</th>
<th>Concentration/dose</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial reverse mutation (Ames test)</td>
<td><em>Salmonella typhimurium</em> TA98, TA100, TA1535, TA1537, TA1538</td>
<td>125, 250, 500, 1 250, 2 500, and 5 000 µg/plate</td>
<td>Negativea</td>
<td>Kramer (1982)</td>
</tr>
<tr>
<td>Point mutation (hgprt locus) assay</td>
<td>Chinese hamster V79 cells</td>
<td>5, 10, 25 and 50 µg/mL</td>
<td>Negativea</td>
<td>Heidemann (1986)</td>
</tr>
<tr>
<td>Chromosomal aberration assay</td>
<td>Chinese hamster V79 cells</td>
<td>4, 25 and 50 µg/mL</td>
<td>Negativea</td>
<td>Heidemann (1985)</td>
</tr>
<tr>
<td>Unscheduled DNA synthesis</td>
<td>Wistar CF HB male rat hepatocytes</td>
<td>1, 3.3, 10, 33.3 and 100 µg/mL</td>
<td>Negative</td>
<td>Müller (1986)</td>
</tr>
<tr>
<td>In vivo mouse micronucleus assay</td>
<td>NMRI mice (both sexes)</td>
<td>5 000 mg/kg bw</td>
<td>Negative</td>
<td>Guenard (1984)</td>
</tr>
<tr>
<td>In vivo DNA covalent binding assay</td>
<td>NMRI male mice</td>
<td>40 mg/kg bw</td>
<td>Negative</td>
<td>Kugler-Steigmeier, Lutz &amp; Schlatter (1988)</td>
</tr>
</tbody>
</table>

bw: body weight; DNA: deoxyribonucleic acid; hgprt: hypoxanthine–guanine phosphoribosyltransferase; S9: 9000 \( \times g \) supernatant fraction from rat liver homogenate

a In the presence and absence of metabolic activation (S9).

### Table 7

**Genotoxicity studies on sisapronil**

<table>
<thead>
<tr>
<th>Test system</th>
<th>Test object</th>
<th>Concentration/dose</th>
<th>Results</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>In vitro</td>
<td><em>Salmonella typhimurium</em> TA98, TA100, TA1535, TA1537; and</td>
<td>15–5 000 µg/plate</td>
<td>Negative; not mutagenic</td>
<td>Cheung (2011)</td>
</tr>
</tbody>
</table>
### Table 8
**Summary of BMD/BMDL results for sisapronil**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sex</th>
<th>BMR</th>
<th>BMD</th>
<th>BMDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSH</td>
<td>M/F</td>
<td>10%</td>
<td>0.921</td>
<td>0.631</td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt;</td>
<td>M/F</td>
<td>10%</td>
<td>2.39</td>
<td>0.786</td>
</tr>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt;</td>
<td>M/F</td>
<td>10%</td>
<td>1.65</td>
<td>0.547</td>
</tr>
<tr>
<td>Liver/body weight</td>
<td>M/F</td>
<td>5%</td>
<td>0.626</td>
<td>0.447</td>
</tr>
<tr>
<td>Thyroid/body weight</td>
<td>M/F</td>
<td>10%</td>
<td>0.919</td>
<td>0.454</td>
</tr>
<tr>
<td>Liver/brain</td>
<td>M/F</td>
<td>10%</td>
<td>1.29</td>
<td>0.448</td>
</tr>
<tr>
<td>Thyroid/brain</td>
<td>M/F</td>
<td>10%</td>
<td>0.486</td>
<td>0.298</td>
</tr>
<tr>
<td>Thyroid hypertrophy</td>
<td>M/F</td>
<td>10%</td>
<td>0.440</td>
<td>0.257</td>
</tr>
<tr>
<td>Thyroid adenoma</td>
<td>M</td>
<td>10%</td>
<td>2.10</td>
<td>0.305</td>
</tr>
</tbody>
</table>

BMD: benchmark dose; BMDL: lower 95% confidence limit on the benchmark dose; BMR: benchmark response; F: females; M: males; T<sub>3</sub>: triiodothyronine; T<sub>4</sub>: thyroxine; TSH: thyroid stimulating hormone  
Source: Boucher (2013)

### Table 9
**BMD<sub>10</sub> and BMDL<sub>10</sub> for hepatocellular hypertrophy<sup>a</sup> in male mice in the carcinogenicity study**

<table>
<thead>
<tr>
<th>Model</th>
<th>BMD&lt;sub&gt;10&lt;/sub&gt; (mg/kg bw per day)</th>
<th>BMDL&lt;sub&gt;10&lt;/sub&gt; (mg/kg bw per day)</th>
<th>P-value</th>
<th>AIC</th>
<th>Scaled residual for Treatment</th>
<th>Scaled residual for Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gamma</td>
<td>1.66</td>
<td>1.22</td>
<td>0.0008</td>
<td>254.8</td>
<td>1.362</td>
<td>−1.861</td>
</tr>
<tr>
<td>Logistic</td>
<td>3.4</td>
<td>2.6</td>
<td>0</td>
<td>263.8</td>
<td>0.798</td>
<td>−3.089</td>
</tr>
<tr>
<td>LogLogistic</td>
<td>0.37</td>
<td>0.082</td>
<td>0.997</td>
<td>243.7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>LogProbit</td>
<td>0.38</td>
<td>0.086</td>
<td>0.850</td>
<td>243.8</td>
<td>0.012</td>
<td>0.012</td>
</tr>
<tr>
<td>Multistage</td>
<td>0.73</td>
<td>0.54</td>
<td>0.248</td>
<td>245.1</td>
<td>−0.45</td>
<td>−0.45</td>
</tr>
<tr>
<td>Multistage-Cancer</td>
<td>1.7</td>
<td>1.2</td>
<td>0.0008</td>
<td>254.8</td>
<td>1.362</td>
<td>−1.861</td>
</tr>
</tbody>
</table>

<sup>a</sup> Precipitation at 50 µg/plate and higher.
<sup>b</sup> Positive controls were sodium nitrite, 9-aminoacridine, 2-nitrofluorene, nitrofurantoin and N-ethyl-N-nitro-nitrosoguanidine in the absence of metabolic activation; and 2-anthramine in the presence of metabolic activation.
<sup>c</sup> Mitomycin C was used as a positive control without metabolic activation, and cyclophosphamide was used as a positive control in the presence of metabolic activation.
<sup>d</sup> Cyclophosphamide was used as a positive control.
<sup>e</sup> The maximum tolerated dose was 250 mg/kg bw. There was a dose-related decrease in the percentage of polychromatic erythrocytes.
Model | BMD₁₀ (mg/kg bw per day) | BMDL₁₀ (mg/kg bw per day) | P-value | AIC | Scaled residual for Treatment | Control
---|---|---|---|---|---|---
Probit | 4.0 | 3.2 | 0 | 265.9 | 0.714 | −3.258
Weibull | 1.7 | 1.2 | 0.000 8 | 254.8 | 1.362 | −1.861
Quantal-Linear | 1.7 | 1.2 | 0.000 8 | 254.8 | 1.362 | −1.861

AIC: Akaike information criterion; BMD₁₀: benchmark dose for a 10% response; BMDL₁₀: lower 95% confidence limit on the benchmark dose for a 10% response.

a Proportion affected: 12/60, 29/60, 46/60 and 56/60 at control, low, middle and high doses, respectively.

Table 10
Susceptibility of representative human intestinal bacteria to avilamycin

<table>
<thead>
<tr>
<th>Bacterial group</th>
<th>High inoculum (1 x 10⁹ cfu/mL)</th>
<th>Low inoculum (1 x 10⁵ cfu/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>MIC₉₀</td>
</tr>
<tr>
<td>Bacteroides fragilis</td>
<td>4–128</td>
<td>8</td>
</tr>
<tr>
<td>Other Bacteroides</td>
<td>4–128</td>
<td>8</td>
</tr>
<tr>
<td>Bifidobacterium</td>
<td>2–128</td>
<td>16</td>
</tr>
<tr>
<td>Clostridium</td>
<td>0.5–8</td>
<td>1</td>
</tr>
<tr>
<td>Enterococcus</td>
<td>2–4</td>
<td>2</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>All &gt;128</td>
<td>&gt;128</td>
</tr>
<tr>
<td>Eubacterium</td>
<td>0.5–128</td>
<td>0.5</td>
</tr>
<tr>
<td>Fusobacterium</td>
<td>0.5–128</td>
<td>4</td>
</tr>
<tr>
<td>Lactobacillus</td>
<td>8–128</td>
<td>16</td>
</tr>
<tr>
<td>Peptostreptococcus</td>
<td>0.062–2</td>
<td>0.25</td>
</tr>
</tbody>
</table>

cfu: colony forming units; MIC: minimum inhibitory concentration
Source: Pridmore (2004a)

Table 11
Summary of ivermectin repeated-dose treatment regimens in selected human clinical efficacy studies in parasitized patients

<table>
<thead>
<tr>
<th>First dose (µg/kg bw)</th>
<th>Subsequent dose(s) (µg/kg bw)</th>
<th>Frequency of treatment</th>
<th>Total no. of doses</th>
<th>Total no. of subjects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>100</td>
<td>Every 2 weeks</td>
<td>6</td>
<td>30</td>
<td>Duke et al. (1991)</td>
</tr>
<tr>
<td>150</td>
<td>150</td>
<td>Monthly</td>
<td>4, 8 or 12</td>
<td>32</td>
<td>Duke et al. (1990)</td>
</tr>
<tr>
<td>150</td>
<td>150</td>
<td>Every 3 months</td>
<td>4, 8 or 11</td>
<td>28</td>
<td>Duke et al. (1992)</td>
</tr>
<tr>
<td>20</td>
<td>200 or 400</td>
<td>Days 1 and 5</td>
<td>2</td>
<td>18</td>
<td>Addiss et al. (1993)</td>
</tr>
<tr>
<td>First dose (µg/kg bw)</td>
<td>Subsequent dose(s) (µg/kg bw)</td>
<td>Frequency of treatment</td>
<td>Total no. of doses</td>
<td>Total no. of subjects</td>
<td>Reference</td>
</tr>
<tr>
<td>----------------------</td>
<td>-------------------------------</td>
<td>-----------------------</td>
<td>--------------------</td>
<td>----------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>20</td>
<td>200 or 400</td>
<td>Days 1 and 5</td>
<td>2</td>
<td>20</td>
<td>Kazura et al. (1993)</td>
</tr>
<tr>
<td>20</td>
<td>200 or 400</td>
<td>Days 1 and 5</td>
<td>2</td>
<td>21</td>
<td>Shenoy et al. (1993)</td>
</tr>
<tr>
<td>20</td>
<td>200 or 400</td>
<td>Days 1 and 5</td>
<td>2</td>
<td>22</td>
<td>Dreyer et al. (1995)</td>
</tr>
<tr>
<td>20</td>
<td>400</td>
<td>Days 1 and 4, then every 2 weeks</td>
<td>13</td>
<td>14</td>
<td>Ismail et al. (1996)</td>
</tr>
<tr>
<td>100</td>
<td>100, then 400</td>
<td>Every 6 months</td>
<td>6 (3 + 3)</td>
<td>92</td>
<td>Nguyen, Mouia-Pelat &amp; Cartel (1996)</td>
</tr>
<tr>
<td>150</td>
<td>400, 600 or 800</td>
<td>Days 1 and 4</td>
<td>2</td>
<td>25</td>
<td>Awadzi et al. (1995, 1999)</td>
</tr>
<tr>
<td>150</td>
<td>400, then 800</td>
<td>Days 1 and ~60–90, then every 12 months</td>
<td>4</td>
<td>172</td>
<td>Gordon et al. (2002)</td>
</tr>
<tr>
<td>150</td>
<td>150 or 400, then 800</td>
<td>Every 3 months</td>
<td>13</td>
<td>319</td>
<td>Gordon et al. (2002)</td>
</tr>
<tr>
<td>800</td>
<td>800</td>
<td>Days 1 and 13</td>
<td>2</td>
<td>12</td>
<td>Awadzi et al. (1999)</td>
</tr>
</tbody>
</table>

Table 12
Studies relevant to risk assessment

<table>
<thead>
<tr>
<th>Species / study type (route of administration)</th>
<th>Doses (mg/kg bw per day)</th>
<th>Critical end-point</th>
<th>NOAEL (mg/kg bw per day)</th>
<th>LOAEL (mg/kg bw per day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>0, 0.1, 0.3, 1.0, 10</td>
<td>Hepatocellular hypertrophy; thyroid follicular cell hypertrophy</td>
<td>1.0</td>
<td>10</td>
</tr>
<tr>
<td>Three-month study of toxicity (gavage)</td>
<td>0, 0.1, 0.3, 1.0, 10</td>
<td>Centrilobular hepatocellular hypertrophy; thyroid follicular cell hypertrophy, hyperplasia and adenoma</td>
<td>0.3</td>
<td>1.0</td>
</tr>
<tr>
<td>One-year study of toxicity (gavage)</td>
<td>0, 0.3, 2.0, 15</td>
<td>Parental toxicity: follicular cell hypertrophy and hyperplasia; increased liver weights</td>
<td>0.3</td>
<td>2.0</td>
</tr>
<tr>
<td>Two-generation reproductive toxicity study (gavage)</td>
<td>0, 0.3, 2.0, 20</td>
<td>Reproductive toxicity: low fertility in males and females, decreased male copulation, decreased female conception indices and decreased ovarian follicle counts in F1 generation</td>
<td>0.3</td>
<td>2.0</td>
</tr>
<tr>
<td>Developmental toxicity study</td>
<td>0, 0.3, 2.0, 20</td>
<td>Offspring toxicity: decreased survival</td>
<td>2.0</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Embryo/fetal toxicity: lower pup weight due to lower body weight gain</td>
<td>2.0</td>
<td>20</td>
</tr>
<tr>
<td>Species / study type (route of administration)</td>
<td>Doses (mg/kg bw per day)</td>
<td>Critical end-point</td>
<td>NOAEL (mg/kg bw per day)</td>
<td>LOAEL (mg/kg bw per day)</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
<td>----------------------------</td>
<td>--------------------</td>
<td>-------------------------</td>
<td>-------------------------</td>
</tr>
<tr>
<td>(gavage) and lower feed consumption in dams</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Rabbit</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Developmental toxicity study (gavage)</td>
<td>0, 0.3, 2.0, 12.5</td>
<td>Maternal toxicity: severe body weight loss, reduced feed intake, moribundity, abortion and premature delivery</td>
<td>2.0</td>
<td>12.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Embryo/fetal toxicity: Reduced pup weight</td>
<td>2.0</td>
<td>12.5</td>
</tr>
<tr>
<td><strong>Dog</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Three-month study of toxicity* (capsule)</td>
<td>0, 0.3, 1, 10</td>
<td>Increased glycogen in hepatocytes; thyroid follicular cell hypertrophy</td>
<td>0.3</td>
<td>1</td>
</tr>
</tbody>
</table>

* A plasma half-life of approximately 100 days was found in a non-GLP-compliant study in dogs.
Annex 3: Template for report item

A sample template for the meeting report item is given below.

It should be noted that the design of the meeting report changed in 2015, resulting in several formatting changes. Text is Times New Roman 12 pt, headings are Arial 11 pt, paper size A4, 1 inch margins, 1.5 line spacing, first paragraph below heading flush left, all subsequent paragraphs indented 0.5 inch, no spacing between paragraphs, paragraphs are fully justified.

WHO template

Report topic: 
Author(s): 
Date: 
Version: 

3.x Compound name [or 4.x, if there are concern forms]

Explanation [prepared by WHO and FAO experts]

(gives general introduction to the compound, if it has been evaluated before, including short description of the conclusions, and why it is on the agenda) (NOTE: to be combined from WHO and FAO parts, preferably before the start of the meeting)

Toxicological and microbiological evaluation [prepared by WHO expert]

(gives short summary of the key data relevant for the evaluation, according to subheadings below, usually identical to “Comments” section of monograph; for antimicrobials, in a separate section, gives a brief summary of relevant data, including full decision-tree evaluation)

Biochemical data

Toxicological data

Microbiological data

Evaluation

(gives the Committee’s conclusions, including establishment of ADI and/or ARfD, and any recommendations with regard to data gaps. Short sentence if monograph has been prepared or addendum)

Residue evaluation [prepared by FAO expert]

Data on pharmacokinetics and metabolism

Residue data
### Analytical methods

### Maximum residue limits

**Summary and conclusions** [prepared by WHO and FAO experts and WHO editor]

**Studies relevant to risk assessment** [example only; species and headings will vary]

<table>
<thead>
<tr>
<th>Species / study type (route of administration)</th>
<th>Doses (mg/kg bw per day)</th>
<th>Critical end-point</th>
<th>NOAEL (mg/kg bw per day)</th>
<th>LOAEL (mg/kg bw per day)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mouse</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eighteen-month study of toxicity and carcinogenicity (diet)</td>
<td>0, x, y, z</td>
<td>Finding</td>
<td>x(^a)</td>
<td>–</td>
</tr>
<tr>
<td><strong>Rat</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Two-year study of toxicity and carcinogenicity (diet)</td>
<td>0, x, y, z</td>
<td>Finding</td>
<td>y</td>
<td>z</td>
</tr>
<tr>
<td>Multigeneration reproductive toxicity study (diet)</td>
<td>0, x, y, z</td>
<td>Reproductive toxicity: Finding</td>
<td>y</td>
<td>z</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Parental toxicity: Finding</td>
<td>x(^*)</td>
<td>y</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Offspring toxicity: Finding</td>
<td>y</td>
<td>z</td>
</tr>
<tr>
<td><strong>Developmental toxicity study (gavage)</strong></td>
<td>0, x, y, z</td>
<td>Maternal toxicity: Finding</td>
<td>–</td>
<td>x(^b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Embryo and fetal toxicity: Finding</td>
<td>x</td>
<td>y</td>
</tr>
<tr>
<td><strong>Rabbit</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Developmental toxicity study (gavage)</td>
<td>0, x, y, z</td>
<td>Maternal toxicity: Finding</td>
<td>–</td>
<td>x(^b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Embryo and fetal toxicity: Finding</td>
<td>x</td>
<td>y</td>
</tr>
<tr>
<td><strong>Dog</strong></td>
<td></td>
<td></td>
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<tr>
<td>Thirteen-week and 1-year toxicity studies(^c) (dietary)</td>
<td>0, x, y, z</td>
<td>Finding</td>
<td>y</td>
<td>z</td>
</tr>
</tbody>
</table>

* Pivotal study value (ref 1)

\(\text{a}\) Highest dose tested.

\(\text{b}\) Lowest dose tested.

\(\text{c}\) Two studies combined.

**Uncertainty factor** (for ADI or ARfD or both) [e.g. 200 (10 for intraspecies variability, 10 for interspecies variability and 2 for use of a LOAEL instead of a NOAEL)]

**Toxicological health-based guidance value** [give health-based guidance value(s) for toxicological effects; e.g. A toxicological ADI of 0–x mg/kg bw could be derived; delete heading if there are no microbiological effects of concern]
**Microbiological health-based guidance value** [give health-based guidance value(s) for microbiological effects; e.g. A microbiological ADI of 0–x mg/kg bw could be derived; delete heading if there are no microbiological effects of concern]

**ADI** (based on toxicological or microbiological effects) [delete toxicological or microbiological, as appropriate]

**ARfD** (based on toxicological or microbiological effects) [delete toxicological or microbiological, as appropriate]

**Residue definition** [to be prepared by FAO]

**MRLs** [to be prepared by FAO]

**Estimated dietary exposure** [to be prepared by FAO]
3.4 Teflubenzuron

**Explanation**

Teflubenzuron (IUPAC name: 1-(3,5-dichloro-2,4-difluorophenyl)-3-(2,6-difluorobenzoyl)urea; CAS no. 83121-18-0) is an insecticide belonging to the benzoylurea group of compounds. Its mode of action is through inhibition of the synthesis of chitin, and hence it is most effective on the developmental stages of insects. Insects are killed as a result of the disruption of their moulting. Teflubenzuron is approved in many countries as an insecticide for use in plant production as well as for control of sea lice (*Lepeophtheirus salmonis* and *Caligus rogercresseyi*) in aquaculture. Teflubenzuron is used as a premix coated onto non-medicated fish feed pellets to achieve an intended dose of 10 mg/kg bw per day for 7 consecutive days. The withdrawal periods range from 7 to 11 days and from 45 to 96 degree-days.

Teflubenzuron has not been previously evaluated by the Committee, although it was evaluated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) as a pesticide in 1994 and 1996 (FAO/WHO, 1995, 1997) and is scheduled for periodic re-evaluation at JMPR’s September 2016 meeting. JMPR established an ADI of 0–0.01 mg/kg bw on the basis of a LOAEL of 2.1 mg/kg bw per day in a mouse carcinogenicity study with the application of an uncertainty factor of 200, including an additional factor of 2 to account for the use of a LOAEL instead of a NOAEL.

The Committee evaluated teflubenzuron at the present meeting at the request of the Twenty-second Session of the Codex Committee on Residues of Veterinary Drugs in Foods (FAO/WHO, 2015), with a view to establishing an acceptable daily intake (ADI) and recommending maximum residue limits (MRLs) in finfish tissues.

**Toxicological and microbiological evaluation**

The Committee considered data on the pharmacokinetics, short- and long-term toxicity, reproductive and developmental toxicity, genotoxicity and carcinogenicity of teflubenzuron. In addition to a sponsor’s submission, relevant studies retrieved from the published literature were evaluated. Most studies submitted by the sponsor were conducted under GLP-compliant conditions. Those that were not conducted under GLP-compliant conditions are identified in this report.

**Biochemical data**

Orally administered teflubenzuron was only partially, but relatively quickly, absorbed. Following a single oral dose of radiolabelled teflubenzuron at 25 mg/kg bw, approximately 20% of the radioactivity was absorbed; only 4% was absorbed when rats were dosed at 750 mg/kg bw, suggesting a dose-dependent absorption (Schlüter, 1986; Hawkins & Mayo, 1988). Peak plasma concentrations were reached within 1–2 hours post-dosing and were maintained at similar levels for up to 8 hours (low dose) or 24 hours (high dose) (Schlüter, 1986). In repeatedly dosed animals, there was some evidence of a dose-dependent plateau in plasma concentration (Ellgehausen et al., 1986).

Most (90–95%) radiolabelled teflubenzuron administered by gavage to rats (single dose at 25 or 750 mg/kg bw or 14 daily doses of 25 mg/kg bw of unlabelled drug followed by a single dose of 25 mg/kg bw radiolabelled drug) was excreted in faeces, primarily as the parent compound. More than 85% of the drug was excreted within the first 24 hours of the dosing. Only a small fraction (0.15–3%) of the total oral dose of teflubenzuron was excreted
in the urine. There was no difference in excretion pattern between sexes or between animals dosed with a single or multiple doses of the drug (Schlüter, 1986). Absorbed teflubenzuron was mostly excreted through bile, predominantly as polar materials. Only negligible residues of teflubenzuron were detected in tissues and organs (< 2% of the dose), with no evidence of accumulation (Schlüter, 1984, 1986; Hawkins & Mayo, 1988).

Metabolites identified in bile and urine were benzoyl or aniline ring hydroxylated teflubenzuron and conjugates of (3,5-dichloro-2,4-difluorophenyl)urea and 3,5-dichloro-2,4-difluoroaniline (Schlüter, 1985; Hawkins & Mayo, 1988). Several polar metabolites were detected in faeces, but the only metabolite characterized was (3,5-dichloro-2,4-difluorophenyl)urea (Schlüter, 1986). Hydrolytic cleavage of the phenylurea bridge was identified as the predominant pathway of teflubenzuron metabolism in a non-GLP-compliant study in which rats were gavaged once with approximately 55 mg/kg bw of the drug. The scission products thus produced were either excreted unmodified or further metabolized and excreted (Koerts et al., 1997).

Toxicological data
Teflubenzuron was shown to have low acute toxicity in laboratory animals. The oral LD$_{50}$ in mice and rats was greater than 5000 mg/kg bw (Ullmann, 1983d,e; Ullmann, Sacher & Vogel, 1988). The dermal LD$_{50}$ in rats was greater than 2000 mg/kg bw (Ullmann, 1983b), and the inhalation LC$_{50}$ in rats was greater than 5000 mg/m$^3$ air (Ullmann, 1983a). Teflubenzuron was not irritating to the skin (Ullmann, 1983g) or eyes (Ullmann, 1983f) of rabbits, and it did not cause skin sensitization in the guinea-pig maximization test (Ullmann, 1984).

Short-term toxicity studies of teflubenzuron in which the drug was administered in diet were conducted in mice (one 13-week study), rats (one 13-week study) and dogs (two 13-week studies and one 52-week study). In all three species, liver was identified as the target organ for toxic effects, as evidenced by elevated enzyme activities and/or adaptive cellular changes.

In a study whose GLP-compliant status could not be verified, mice were administered teflubenzuron at a concentration of 0, 100, 1000 or 10 000 mg/kg diet (equal to 0, 12, 115 and 1213 mg/kg bw per day for males and 0, 14, 142 and 1450 mg/kg bw per day for females, respectively) for 13 weeks. In the high-dose group, activities of liver enzymes (alkaline phosphatase in males, alanine transaminase in females) and total cholesterol levels (females) were elevated, and blood glucose levels (both sexes) were altered. In the mid- and high-dose groups, absolute and relative liver weights were increased, with centrilobular hepatocellular swelling (both sexes) and microscopic fatty changes (in males). The NOAEL was identified as 100 mg/kg feed (equal to 12 mg/kg bw per day) (Takahashi et al., 1987). Rats were administered teflubenzuron at a concentration of 0, 100, 1000 or 10 000 mg/kg feed (equal to 0, 8, 82 and 809 mg/kg bw per day for males and 0, 9, 94 and 942 mg/kg bw per day for females, respectively) for 13 weeks (Suter et al., 1987c). Some animals from the control and high-dose groups were observed for an additional 4 weeks without treatment. Increased activities of several enzymes, notably aspartate transaminase (middle and high doses, both sexes), ornithine transcarbamylase (high dose, both sexes), alanine transaminase (mid- and high-dose males), lactate dehydrogenase (high-dose males) and alkaline phosphatase (all treated males), were observed, which returned to normal levels after 4 weeks of drug withdrawal. At necropsy, increased absolute and relative weights of liver in females and of testes in males were observed at the high dose. The statistically significant change in alkaline phosphatase activity in male rats of the low-dose group was not considered to be biologically relevant, being less than 1.5-fold above the control value and within the normal physiological range. Based on effects on liver enzymes at 1000 mg/kg feed (equal to
82 mg/kg bw per day), a NOAEL of 100 mg/kg feed (equal to 8 mg/kg bw per day) was identified.

In a non-GLP-compliant study, the potential of teflubenzuron to form methaemoglobin and exert other effects on haematological parameters in rats was investigated in a comparative study that investigated five benzoylurea insecticides (Tasheva & Hristeva, 1993). Although reticulocyte count was increased in rats treated with teflubenzuron (100 mg/kg bw per day for 28 days), it was not associated with anaemia or the formation of methaemoglobin.

In dogs administered teflubenzuron at a concentration of 0, 100, 1000 or 10 000 mg/kg feed (equal to 0, 3.5, 33.7 and 318.2 mg/kg bw per day for males and 0, 4.0, 42.8 and 417.1 mg/kg bw per day for females, respectively) for 13 weeks, activities of alanine transaminase, aspartate transaminase, alkaline phosphatase and ornithine transcarbamylase were increased in both sexes at the high dose. At necropsy, absolute and relative liver weights were elevated and the incidence of nodular foci in the pyloric or fundic region of the stomach was increased at the high dose. Isolated dark red foci were noted in the pyloric region of the stomach at the middle and high doses, and follicular hyperplasia of the pyloric mucosa was noted in most animals at the high dose. Mild hepatitis in one male and centrilobular hepatic necrosis in another male were observed at the low dose. Also, moderate chronic active hepatitis was diagnosed in one animal of each sex at the high dose. Given the lack of a clear dose–response relationship for hepatitis, the mild microscopic hepatic changes noted in two dogs in the low-dose group were not considered to be a treatment-related adverse effect. The NOAEL was 100 mg/kg feed (equal to 3.5 mg/kg bw per day), based on stomach lesions at 1000 mg/kg feed (equal to 33.7 mg/kg bw per day) (Bathe et al., 1985).

In a supplementary study to clarify the effects on liver, dogs were administered teflubenzuron at a concentration of 0, 30 or 100 mg/kg feed (equal to 0, 1.2 and 4.4 mg/kg bw per day for males and 0, 1.5 and 5.1 mg/kg bw per day for females, respectively) for 13 weeks. There were no treatment-related adverse effects identified in clinical examination, laboratory testing, and macroscopic or microscopic examination of organs. The NOAEL was 100 mg/kg feed (equal to 4.4 mg/kg bw per day), the highest dose tested (Bathe et al., 1987).

In a 52-week study (Sachsse et al., 1986), dogs were administered teflubenzuron at a concentration of 0, 30, 100 or 500 mg/kg feed (equal to 0, 1.0, 3.2 and 17.3 mg/kg bw per day for males and 0, 1.2, 4.0 and 18.0 mg/kg bw per day for females, respectively). At necropsy, the absolute liver weight in males was increased at the high dose, but no treatment-related changes were noted in gross pathological or histopathological examinations. The NOAEL was 100 mg/kg feed (equal to 3.2 mg/kg bw per day), based on the liver weight change at 500 mg/kg feed (equal to 17.3 mg/kg bw per day).

The Committee identified an overall NOAEL of 100 mg/kg feed (equal to 4.4 mg/kg bw per day) from three short-term studies in dogs, based on the findings of adverse effects in liver at a dose of 500 mg/kg feed (equal to 17.3 mg/kg bw per day).

In a carcinogenicity study in mice, teflubenzuron was administered at a concentration of 0, 15, 75 or 375 mg/kg feed (equal to 0, 2.1, 10.5 and 53.6 mg/kg bw per day for males and 0, 3.1, 15.4 and 71.7 mg/kg bw per day for females, respectively) for 78 weeks, with an interim kill at week 52. Aspartate transaminase, alanine transaminase, ornithine transcarbamylase, lactate dehydrogenase and alkaline phosphatase activities were elevated in high-dose males, but only alanine transaminase activity was elevated in high-dose females. Absolute and relative liver weights were higher in both sexes at the high dose, and relative liver weight was slightly increased in the mid-dose males. The incidence of macroscopic hepatic nodules was increased in the high-dose males. Histopathology indicated an increased incidence of hepatocellular adenomas and nodular hepatic hyperplasia in males treated at the
middle and high doses compared with both concurrent and historical controls, but there was no difference in the incidence of hepatic carcinoma. Several treatment-related, dose-dependent, non-neoplastic hepatic changes were also observed, which were more pronounced in males than in females. In particular, males in the control, low-dose, mid-dose and high-dose groups, respectively, had dose-dependent incidences of hepatocellular hypertrophy (12/60, 29/60, 46/60 and 56/60), single-cell necrosis (13/60, 26/60, 42/60 and 56/60), phagocytic cell foci (17/60, 21/60, 43/60 and 54/60) and lipofuscin accumulation (8/60, 11/60, 20/60 and 27/60). In the low-dose group, the incidence, but not the severity, of these non-neoplastic hepatic changes was significantly higher when compared with the controls (Suter et al., 1987b).

Histopathological sections of liver from male mice in this study were re-evaluated by an independent pathologist, with a focus on nodular liver lesions. The pathologist concluded that there was a dose-related increase in the incidence of hepatocellular hyperplastic nodules and a slight, but statistically non-significant, increase in hepatocellular adenoma (Vesselinovitch, 1988).

Given that only hepatic adenomas were observed and that the genotoxicity test results were negative (see below), the Committee considered that teflubenzuron was not carcinogenic in mice. However, the Committee concluded that teflubenzuron induced hyperplastic proliferation in liver of mice by an unknown mechanism. Based on the increased incidence of non-neoplastic hepatic changes observed in liver (e.g. hepatocellular hypertrophy, single-cell necrosis, phagocytic cell foci, lipofuscin accumulation) at all doses, no NOAEL could be identified. The lowest dietary concentration, 15 mg/kg feed (equal to 2.1 mg/kg bw per day), was identified as the LOAEL.

In the absence of a NOAEL, to better characterize the point of departure, the Committee conducted a dose–response analysis of these data using the benchmark dose (BMD) approach. Of several non-neoplastic hepatic changes identified, hepatocellular hypertrophy was considered to be the most toxicologically relevant effect for dose–response modelling. The BMD and a lower confidence limit on the benchmark dose for a 10% response over the controls (BMDL_{10}) were determined using nine different dichotomous models. Three models (LogLogistic, LogProbit and Multistage) provided acceptable fits based on statistical considerations. However, the BMD and BMDL_{10} estimated by the LogLogistic and LogProbit models were much lower than the lowest dose used in the study. Furthermore, the Multistage model provided a better fit of the BMDL value for the benchmark response at the low end of the observed range of the data. Therefore, the Committee considered the BMDL_{10} of 0.54 mg/kg bw per day for the BMD of 0.73 mg/kg bw per day estimated by the Multistage model as the most appropriate point of departure for this study.

In a 120-week carcinogenicity study, rats were administered teflubenzuron at a concentration of 0, 20, 100 or 500 mg/kg feed (equal to 0, 1.0, 4.8 and 24.8 mg/kg bw per day for males and 0, 1.2, 5.9 and 29.9 mg/kg bw per day for females, respectively) for 120 weeks, with an interim kill at weeks 53 and 107. Mortality ranged from 40% to 50% at week 120, which was not influenced by treatment. Increased (approximately 1.5- to 3-fold) activities of alanine transaminase, aspartate transaminase and ornithine transcarbamylase were noted in males in the high-dose group. Absolute and relative liver weights were increased in high-dose males. Several non-neoplastic microscopic changes were noted in different organs, but were not treatment related. Trend analysis identified increased incidences of haemangiomas in mesenteric lymph nodes and pancreatic exocrine carcinoma in the high-dose males. However, they were not significantly different when compared with historical controls. Also, the occurrence of pancreatic exocrine carcinoma was too infrequent (2/47 versus 0/50) to allow a meaningful comparison to be drawn. Based on the effect on liver enzymes and liver weight at
500 mg/kg feed (equal to 24.8 mg/kg bw per day), the NOAEL was 100 mg/kg feed (equal to 4.8 mg/kg bw per day) (Suter et al., 1987a).

In a supplemental carcinogenicity study (Tennekes et al., 1989), rats were administered teflubenzuron at a concentration of 0, 2500 or 10 000 mg/kg feed (equal to 0, 122.5 and 487.3 mg/kg bw per day for males and 0, 154 and 615.2 mg/kg bw per day for females, respectively) for 111 weeks, with an interim kill at week 104. Clinical biochemistry revealed increased activities of alanine transaminase and aspartate transaminase in males at both doses and of aspartate transaminase in females at the high dose. The absolute and relative liver (interim and terminal kill) and kidney (interim kill) weights were increased in males at the high dose compared with controls. Dose-dependent increases in the incidence of diffuse, clay-coloured discoloration and focal and multifocal discoloration of livers were observed in treated males (both doses). Also, treatment-related non-neoplastic microscopic changes (e.g. fatty changes, mixed cell and basophilic cell foci, focal hepatocellular hyperplasia, spongiosis hepatitis) were noted in the liver of both sexes at both doses tested, lesions being more severe in males than in females. There was no compound-related increase in the incidence of any tumours observed in this study, including mesenteric lymph node haemangioma and pancreatic exocrine carcinoma in male rats, thus confirming the lack of association between substance administration and the occurrence of these tumours suggested from the previous study.

Although no NOAEL could be identified in the second study owing to non-neoplastic microscopic hepatic changes and elevated liver enzyme activities in both treatment groups, the Committee was able to identify an overall NOAEL of 100 mg/kg feed (equal to 4.8 mg/kg bw per day) from the two chronic toxicity and carcinogenicity studies in rats.

The Committee concluded that teflubenzuron is not carcinogenic in mice or rats.

The genotoxic potential of teflubenzuron was investigated in an adequate range of in vitro and in vivo assays. No evidence of genotoxicity was detected, and teflubenzuron was considered unlikely to be genotoxic.

In view of the lack of genotoxicity and the absence of carcinogenicity in mice and rats, the Committee concluded that teflubenzuron is unlikely to pose a carcinogenic risk to humans.

In a multigeneration reproductive toxicity study, teflubenzuron was administered to rats at a concentration of 0, 20, 100 or 500 mg/kg feed (equal to 0, 1.5, 7.4 and 36.9 mg/kg bw per day for males and 0, 1.6, 7.9 and 39.5 mg/kg bw per day for females, respectively). The only treatment-related adverse effect noted was a significant increase in the incidence of unilateral and bilateral dilatation of the renal pelvis in F₁ pups in the high-dose group (6.8%) when compared with the controls (0.9%). No such effect was seen in the F₂ generation. The NOAEL for offspring toxicity was 100 mg/kg feed (equal to 7.4 mg/kg bw per day), and the NOAEL for both parental and reproductive toxicity was 500 mg/kg feed (equal to 36.9 mg/kg bw per day), the highest dose tested (Osterburg, 1989).

The developmental toxicity of teflubenzuron was investigated in pregnant rats by gavage administration at 0, 10, 50 or 250 mg/kg bw per day from days 6 to 15 of gestation. The number of live pups per dam was significantly reduced at the high dose when compared with the controls. The NOAEL for maternal toxicity was 250 mg/kg bw per day, the highest dose tested, and the NOAEL for embryo/fetal toxicity was 50 mg/kg bw per day, based on a reduction in the number of live pups per dam at 250 mg/kg bw per day (Gleich, Weisse & Unkelbach, 1986).

In a second developmental toxicity study, teflubenzuron was administered by gavage to pregnant rats at 0, 100, 300 or 1000 mg/kg bw per day during days 7–17 of gestation. No treatment-related toxicity was observed in dams, for both general health and reproductive parameters. No external, visceral or skeletal abnormalities were observed in pups. The
NOAEL for both maternal and embryo/fetal toxicity was 1000 mg/kg bw per day, the highest dose tested (Ishida et al., 1987).

An overall NOAEL of 1000 mg/kg bw per day for maternal toxicity was identified. No overall NOAEL for embryo/fetal toxicity could be identified, as the reason for the difference in NOAELs for embryo/fetal toxicity in these two developmental toxicity studies in rats was unknown.

Pregnant rabbits were dosed with teflubenzuron by gavage at 0, 10, 50 or 250 mg/kg bw per day from days 6 to 18 of gestation and killed on day 29 of gestation. No maternal or reproductive toxicity was observed, and there were no developmental abnormalities. The only significant effect noted in the offspring was decreased survival during the first 24 hours in the high-dose group (88.5%) compared with the controls (100%). The NOAEL for embryo/fetal toxicity was 50 mg/kg bw per day, based on decreased survival at 250 mg/kg bw per day, and the NOAEL for maternal toxicity was 250 mg/kg bw per day, the highest dose tested (Gleich et al., 1985).

To further elucidate the embryotoxic effect in rabbits, a small supplementary study (five per group) was conducted by administering teflubenzuron by gavage at 0, 250, 500 (killed on day 19) or 500 mg/kg bw per day (killed at day 29) on days 6–18 of gestation. There was evidence of embryotoxicity in all treated animals, although no maternal toxicity was identified (Gleich, 1985).

In another developmental toxicity study in rabbits, pregnant animals were dosed with teflubenzuron by gavage at 0 or 1000 mg/kg bw per day during days 6–18 of pregnancy and killed on gestation day 28. Treated rabbits had a higher incidence of liver lesions compared with controls, but there were no treatment-related reproductive or developmental abnormalities. No NOAEL was identified for maternal toxicity, as effects were noted at the only dose tested. The NOAEL for embryo/fetal toxicity was 1000 mg/kg bw per day, the only dose tested. However, this study did not evaluate offspring survival during the first 24 hours, and hence the Committee cannot discount the effects seen in the previous study (Osterburg, 1987).

An overall NOAEL of 500 mg/kg bw per day was identified for maternal toxicity, and an overall NOAEL of 50 mg/kg bw per day was identified for embryo/fetal toxicity.

**Microbiological data**
Considering the chemical structure and mode of action of teflubenzuron, the Committee did not anticipate any adverse effects of teflubenzuron residues on human gastrointestinal microbiota.

**Evaluation**
An ADI of 0–5 µg/kg bw was established on the basis of a BMDL$_{10}$ of 0.54 mg/kg bw per day for hepatocellular hypertrophy in male mice observed in the carcinogenicity study, with application of an uncertainty factor of 100 to account for interspecies and intraspecies variability, and rounded to one significant figure.

The use profile of teflubenzuron as a veterinary drug is such that dietary exposure to teflubenzuron from a large portion is unlikely to be markedly greater than that from chronic consumption. The toxicological profile of teflubenzuron is such that it is unlikely to present an acute hazard. The Committee therefore concluded that it was not necessary to assess the acute risk from exposure to teflubenzuron when used as a veterinary drug.

A toxicological monograph was prepared.

**Residue evaluation** [prepared by FAO]
## Summary and conclusions

**Studies relevant to risk assessment**

<table>
<thead>
<tr>
<th>Species/study type (route)</th>
<th>Dose (mg/kg bw per day)</th>
<th>Critical end-point</th>
<th>NOAEL (mg/kg bw per day)</th>
<th>LOAEL (mg/kg bw per day)</th>
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<tbody>
<tr>
<td><strong>Mouse</strong></td>
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<tr>
<td>Eighteen-month carcinogenicity study (dietary)</td>
<td>0, 2.1, 10.5, 53.6</td>
<td>Hepatocellular adenoma</td>
<td>2.1</td>
<td>10.5</td>
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<td></td>
<td></td>
<td>Hepatocellular hypertrophy</td>
<td>–</td>
<td>2.1(^a)</td>
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<td></td>
<td></td>
<td><strong>BMDL(_{10}): 0.54(^*)</strong></td>
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<tr>
<td><strong>Rat</strong></td>
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<td>Two-year toxicity and carcinogenicity study (dietary)</td>
<td>0, 1.0, 4.8, 24.8</td>
<td>Increased activity of liver enzymes, and increased liver weights in males</td>
<td>4.8(^b)</td>
<td>24.8(^c)</td>
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<td>Two-generation reproductive toxicity study (dietary)</td>
<td>0, 1.5, 7.4, 36.9</td>
<td>Unilateral and bilateral dilatation of the renal pelvis in F(_{1}) pups</td>
<td>Offspring toxicity: 7.4</td>
<td>36.9</td>
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<td></td>
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<td>Parental toxicity: 36.9(^d)</td>
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<td>Reproductive toxicity: 36.9(^d)</td>
<td>–</td>
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<tr>
<td>Developmental toxicity (gavage)</td>
<td>Study 1: 0, 10, 50, 250</td>
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<td>Study 2: 0, 100, 300, 1 000</td>
<td>Decreased number of live pups per dam</td>
<td>Embryo/fetal toxicity: 50 (study 1)</td>
<td>250 (study 1)</td>
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<td>Maternal toxicity: 1 000(^b,d)</td>
<td>–</td>
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<tr>
<td><strong>Rabbit</strong></td>
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<tr>
<td>Developmental toxicity (gavage)</td>
<td>Study 1: 0, 10, 50, 250</td>
<td>Decreased survival of offspring within 24 h of birth</td>
<td>Embryo/fetal toxicity: 50(^b)</td>
<td>250(^c)</td>
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<tr>
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<td>Study 2: 0, 250, 500</td>
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<td>Maternal toxicity: 500(^b,d)</td>
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<td>Study 3: 0, 1 000</td>
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<tr>
<td><strong>Dog</strong></td>
<td></td>
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<tr>
<td>Thirteen-week study of toxicity (dietary)</td>
<td>Study 1: 0, 3.5, 33.7, 318.2</td>
<td>Increased liver weight</td>
<td>4.4(^b)</td>
<td>17.3(^d)</td>
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<tr>
<td>One-year study of toxicity (dietary)</td>
<td>0, 1.0, 3.2, 17.3</td>
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</table>

\(^*\) Critical point of departure for ADI (Suter et al., 1987b).

\(^a\) Lowest dose tested.

\(^b\) Overall NOAEL.

\(^c\) Overall LOAEL.

\(^d\) Highest dose tested.
Uncertainty factor
100 (10 for interspecies and 10 for intraspecies variability)

ADI (based on toxicological effects)
0–5 µg/kg bw

Residue definition [prepared by FAO]

MRLs [prepared by FAO]

Estimated dietary exposure [prepared by FAO]

3.5 Lasalocid sodium

Explanation
Lasalocid sodium (CAS No. 25999-20-6) is produced by *Streptomyces lasaliensis* and is a mixture of several closely related homologues: A, B, C, D and E. Lasalocid homologues B, C, D and E make up no more than a total of 10% of the total weight of the active substance.

Lasalocid sodium, a divalent polyether ionophore antibiotic, is approved for continuous use to control coccidiosis in poultry species at concentrations of 7.5–125 mg/kg feed. It is approved to protect against *Eimeria* species in broilers and replacement pullets, turkeys, pheasants and quails.

The mechanism of action of lasalocid and other ionophores has been extensively investigated and reported. Like other carboxylic polyether ionophores, lasalocid disturbs ionic homeostasis, leading to osmotic lysis of coccidia.

Lasalocid sodium has not previously been evaluated by the Committee. The Committee evaluated lasalocid sodium at the present meeting at the request of the Twentieth Session of CCRVDF (2) with a view to establishing an ADI and recommending MRLs in poultry tissues and eggs.

Toxicological and microbiological evaluation
The Committee considered data on pharmacodynamics, pharmacokinetics, short-term and long-term toxicity, reproductive and developmental toxicity, genotoxicity, carcinogenicity and microbiological safety. In addition to the sponsor’s submission, a number of studies were retrieved from the published literature. Although most of the studies submitted to the Committee pre-date GLP implementation, the overall package of data was sufficient to allow the derivation of a robust ADI. Those studies that were not performed to GLP standards are identified in this report.

Biochemical data
Following oral administration of a single radiolabelled dose of lasalocid sodium to mice, radioactivity was rapidly absorbed and excreted. Peak concentrations of radiolabelled material were seen in whole blood 15 minutes after administration, and levels had declined to background within 24 hours. The half-life of elimination of radioactivity in whole blood was 3 hours. Radioactivity was widely distributed to tissues, with the highest concentrations seen

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9 This example has been included to illustrate a veterinary drug for which a microbiological health-based guidance value has been derived. Note, however, that this meeting report item was published before the current requirement for full referencing.
in liver, where they peaked 1 hour after administration. Approximately 95% of radioactivity was excreted in the faeces, and approximately 1% in urine, within 24 hours. A similar pattern was seen following multiple oral administrations, with radioactivity peaking in whole blood 30 minutes after the last dose and declining to background levels by 24 hours. Tissue levels were highest in the liver, where they remained detectable 48 hours after administration. Seventy-seven per cent of radioactivity was excreted in faeces within 4 hours of the last dose, and 95% within 24 hours. Excretion was observed to be more rapid in female mice than in male mice, with radioactivity in faeces peaking between 4 and 8 hours in females and between 8 and 12 hours in males.

The pattern of pharmacokinetic behaviour in rats following a single oral administration of radiolabelled lasalocid sodium was comparable to that seen in mice, with rapid absorption and excretion and a wide distribution of radioactivity in tissues. Whole blood radioactivity peaked at 3 hours, and the half-life of elimination was 4.8 hours. Radioactivity was widely distributed to tissues, with the highest levels seen in the liver, where it peaked at approximately 6 hours after administration. Approximately 85% of the administered dose was excreted in faeces within 24 hours, and approximately 1% was excreted in urine over the same period. Similar results were seen after seven daily oral doses. In bile duct–cannulated male rats administered a single oral dose of radiolabelled lasalocid, approximately 63% of the dose was absorbed. Approximately 59% of the dose was excreted in bile within 48 hours.

In a comparative metabolism study in pig, dog, rat, mouse, chicken and turkey, the radioactive metabolite profile was similar in the faecal and liver fractions, although the relative proportions varied. The only component identified was lasalocid A, which represented the major component of the total radioactive residues in the faeces and liver in all species.

Although other residues were not identified, they were present at low levels and are considered to be minor.

Toxicological data
The acute toxicity of lasalocid sodium has been investigated in a number of species. Oral LD$_{50}$ values were 146, 122, 33 and 40 mg/kg bw in the mouse, rat, neonatal rat and rabbit, respectively. The increased sensitivity of the rabbit may be due to the increased sensitivity of this species to effects of antimicrobial drugs on the intestinal microflora.

Lasalocid sodium was not irritating to the skin of rabbits but caused corneal irritation, conjunctival redness and chemosis when applied to the eyes.

Lasalocid sodium did not cause skin sensitization in the guinea-pig maximization test.

In a non-GLP 13-week study in rats, lasalocid sodium was administered in the diet at concentrations adjusted to achieve doses of 0, 2, 5 and 20 mg/kg bw per day. Based on reduced feed consumption, increased liver to body weight ratios and increased haemosiderin in the liver in females, the LOAEL was 5 mg/kg bw per day, and the NOAEL was 2 mg/kg bw per day.

In a non-GLP 13-week study in weanling rats, lasalocid sodium was administered in the diet at concentrations adjusted to achieve doses of 0, 1, 2, 3 and 10 mg/kg bw per day. Based on increased alkaline phosphatase levels seen in males at all doses at week 13, the LOAEL was 1 mg/kg bw per day. No NOAEL could be established. It is noted, however, that the low-dose effect on alkaline phosphatase seen in this study was not seen in other rat studies.

In a non-GLP 13-week study performed in weanling rats that had been exposed to lasalocid sodium in utero (parents were exposed prior to and during mating, gestation and lactation), the substance was administered in the diet at concentrations adjusted to achieve
doses of 0, 1, 2, 3 and 10 mg/kg bw per day. Based on increased haemosiderin seen in the liver of males and (predominantly) females, the LOAEL was 3 mg/kg bw per day, and the NOAEL was 2 mg/kg bw per day.

In a non-GLP 13-week toxicity study in dogs, lasalocid sodium was administered in gelatine capsules at doses of 0, 2, 5 and 10 mg/kg bw per day. Transient muscle weakness involving primarily the hindlimbs was noted in animals at the top dose only. Based on decreased serum chloride levels, increased spleen weights, increased congestion in the spleen and increased hepatocyte vacuolation, the LOAEL was 5 mg/kg bw per day, and the NOAEL was 2 mg/kg bw per day.

In a 2-year toxicity study in dogs, lasalocid sodium was administered in the diet at concentrations of 0, 10, 35 and 180 mg/kg feed (equivalent to 0, 0.25, 1 and 5 mg/kg bw per day, respectively). Based on the transient intermittent paralysis of limbs occurring on a single day and on increased alkaline phosphatase levels, the LOAEL was 180 mg/kg feed (equivalent to 5 mg/kg bw per day), and the NOAEL was 35 mg/kg feed (equivalent to 1 mg/kg bw per day).

In a 24-month carcinogenicity study, mice were administered lasalocid sodium in feed at a concentration of 0, 10 (low-dose animals were dosed with 20 mg/kg feed for the first 5 weeks of the study, after which the dose was adjusted downward), 35 (mid-dose animals were dosed with 60 mg/kg feed for the first 5 weeks, after which the dose was adjusted downward) or 120 mg/kg feed (equivalent to 0, 1.5, 5.25 and 18 mg/kg bw per day after week 5). Lasalocid sodium did not show evidence of tumorigenic potential. The NOAEL was 120 mg/kg feed (equal to 8.1 mg/kg bw per day), the highest dose tested.

In a 30-month toxicity and carcinogenicity study, rats were administered lasalocid sodium in feed at a concentration of 0, 10, 35 or 120 mg/kg (equal to mean doses of 0, 0.5, 1.8 and 6.2 mg/kg bw per day for males and 0, 0.6, 2.2 and 8.1 mg/kg bw per day for females, respectively). The animals used in this study were weanlings bred from parental animals administered the same doses of lasalocid sodium during breeding, gestation and lactation. Lasalocid sodium did not demonstrate tumorigenic properties in this study. Based on a transient impairment of righting and grasping reflexes seen in females between weeks 31 and 49, the LOAEL was 120 mg/kg feed (equal to 8.1 mg/kg bw per day), the highest dose tested.

Lasalocid sodium did not show evidence of genotoxic potential in a range of in vitro tests covering the end-points of gene mutation and chromosomal aberration. Although there was no in vivo test for chromosomal effects, the Committee considered that this was unnecessary in view of the existing genotoxicity and carcinogenicity data.

In a multigeneration reproductive toxicity study incorporating a teratology arm, rats were administered lasalocid sodium in feed at a concentration of 0, 10, 35 or 120 mg/kg (equivalent to 0, 0.5, 1.75 and 6 mg/kg bw per day). At weaning, F₁ animals were randomly selected to become the parents of the F₂ generation; at weaning of F₂ animals, these were randomly selected to become parents of the F₃ generation. F₀ animals and F₂ animals were mated more than once in order to allow for evaluations of teratology. A number of effects were noted in parental animals in the high-dose group: effects on body weight and feed consumption, decreased pregnancy and fertility rates, and decreased mean numbers of corpora lutea and implantations per pregnant dam. The mean numbers of corpora lutea and implantations per pregnant dam were also reduced in the mid-dose group. The LOAEL for maternal effects was therefore 35 mg/kg feed (equivalent to 1.75 mg/kg bw per day), and the NOAEL for maternal effects was 10 mg/kg feed (equivalent to 0.5 mg/kg bw per day). A number of developmental toxicity effects were seen at the high dose: fetal body weights were slightly reduced, the incidence of visceral and skeletal variants was increased, the number of pups surviving to weaning was decreased and body weights of pups surviving until weaning
were decreased. The highest dose of 120 mg/kg feed (equivalent to 6 mg/kg bw per day) was therefore the LOAEL for developmental toxicity, and the NOAEL was 35 mg/kg feed (equivalent to 1.75 mg/kg bw per day).

In a developmental toxicity study in rabbits, lasalocid sodium was administered by oral gavage over days 6–28 of gestation at a dose of 0, 0.5, 1 or 2 mg/kg bw per day. A NOAEL for maternal effects could not be established, as soft stools and effects on body weight gain and feed consumption were seen at all doses. This is likely the result of the known sensitivity of rabbits to antibacterial effects on the microflora of the gastrointestinal tract, and consequently it is not considered appropriate to consider the maternal toxicity in relation to the derivation of an ADI. The LOAEL for developmental effects was 1 mg/kg bw per day, based on decreased litter weights, increased incidence of forelimb flexure and minor skeletal abnormalities/variants at this dose. Although the Committee acknowledges the possibility that these effects may have been secondary to maternal toxicity, it considers the NOAEL for developmental toxicity to be 0.5 mg/kg bw per day.

No original studies dedicated specifically to the evaluation of the neurotoxic potential of lasalocid sodium were provided. Literature data indicate that polyether ionophores, including lasalocid, do have neurotoxic potential. In line with this, a number of the repeated-dose studies summarized above did include examination of neurological end-points. Evidence of neurotoxicity, consisting of transient patterns of muscle weakness involving primarily the hindlimbs, was seen in the 13-week and 2-year dog studies. These effects were seen only at the highest dose and resolved spontaneously, despite continued administration of the drug. In addition, in the 30-month rat study, impairment of the righting and grasping reflexes was seen. A clear effect was evident only at the top dose and, as with the effects seen in the dog, resolved spontaneously, despite continued administration of the drug.

No observations in humans were identified.

**Microbiological data**

A JECFA decision-tree approach that was adopted by the sixty-sixth meeting of the Committee (Annex 1, reference 181) and which complies with VICH Guideline 36 (GL36) (13) was used by the Committee to determine the need for, and to establish, if necessary, a microbiological ADI for lasalocid sodium. Studies of microbiological activity against bacterial strains representative of the human colonic flora were evaluated.

The microbiological ADI was derived from in vitro minimum inhibitory concentration (MIC) data as described in VICH GL36. The strains needed to determine the MIC_{calc}, which is the minimum inhibitory concentration derived from the lower 90% confidence limit for the mean minimum concentration required to inhibit the growth of 50% of organisms (MIC_{50}) of the relevant genera for which the drug is active, were chosen according to these guidelines, which state that an intrinsically resistant bacterial genus should not be included. The genera with a MIC_{50}, including *Eubacterium* (0.125 μg/mL), *Bacteroides* (32 μg/mL), *Bifidobacterium* (0.25 μg/mL), *Fusobacterium* (1 μg/mL), *Peptostreptococcus* (2 μg/mL), *Clostridium* (0.125 μg/mL), *Enterococcus* (0.5 μg/mL) and *Lactobacillus* (0.125 μg/mL), were used to determine the MIC_{calc}.

Lasalocid sodium residues may be present at low levels in meat products consumed by humans; therefore, lasalocid sodium–related residues could enter the colon of a person ingesting edible tissues from treated animals. The Committee used pharmacokinetic studies and faecal binding studies to determine the fraction of the oral dose available to the human intestinal microflora. Lasalocid sodium was poorly absorbed after oral administration in animals and also binds extensively (> 90%) to faecal contents. Therefore, low levels of lasalocid sodium residues entering the human colon will remain biologically active. There is potential for disruption of the colonization barrier in the human gastrointestinal tract, as MIC
values for some of the most relevant and predominant genera in the gastrointestinal tract were susceptible to lasalocid sodium. Lasalocid sodium does not appear to select for resistance in bacteria, and carboxylic polyether ionophores are not used in human medicine.

The formula for calculating the microbiological ADI is as follows:

\[
\text{Upper bound of the ADI (μg/kg bw)} = \frac{\text{MIC}_{\text{calc}} \times \text{Mass of colon content}}{\text{Fraction of oral dose available to microorganisms} \times \text{Body weight}}
\]

The equation terms are derived as described below.

\(\text{MIC}_{\text{calc}}\): In accordance with Appendix C of VICH GL36, calculation of the estimated no-observed-adverse-effect concentration (NOAEC) (MIC\text{calc}) for colonization barrier disruption uses MIC values from the lower 90% confidence limit of the mean MIC\text{50} for the most relevant and sensitive human colonic bacterial genera. The strains needed to determine the MIC\text{calc} were chosen according to these guidelines, which state that an intrinsically resistant bacterial genus should not be included. Based on the MIC\text{50} values for \textit{Eubacterium} (0.125 μg/mL), \textit{Bacteroides} (32 μg/mL), \textit{Bifidobacterium} (0.25 μg/mL), \textit{Fusobacterium} (1 μg/mL), \textit{Peptostreptococcus} (2 μg/mL), \textit{Clostridium} (0.125 μg/mL), \textit{Enterococcus} (0.5 μg/mL) and \textit{Lactobacillus} (0.125 μg/mL), the MIC\text{calc} is 0.228 μg/mL.

\(\text{Mass of colon content}\): A value of 220 g is based on the colon content measured from humans.

\(\text{Fraction of oral dose available to microorganisms}\): It is recommended that the fraction of an oral dose available for colonic microorganisms be based on in vivo measurements for the drug administered orally. Alternatively, if sufficient data are available, the fraction of the dose available for colonic microorganisms can be calculated as 1 minus the fraction (of an oral dose) excreted in urine. Human data are encouraged, but, in their absence, non-ruminant animal data are recommended. In the absence of data to the contrary, it should be assumed that metabolites have antimicrobial activity equal to that of the parent compound. The fraction may be lowered if the applicant provides quantitative in vitro or in vivo data to show that the drug is inactivated during transit through the intestine. Lasalocid sodium is poorly absorbed and is excreted in faeces of experimental animals, primarily in unchanged form. Lasalocid sodium binds rapidly and extensively (>90%) to faecal contents; therefore, the fraction of oral dose available to microorganisms would be 0.10.

\(\text{Body weight}\): The body weight of an adult human is assumed to be 60 kg.

The upper bound of the microbiological ADI for lasalocid sodium is therefore calculated as follows:

\[
\text{Upper bound of the ADI} = \frac{0.228 \, \mu g/mL \times 220 \, g}{0.10 \times 60 \, \text{kg bw}} = 8.4 \, \mu g/kg \, \text{bw}
\]

Therefore, a microbiological ADI of 0–8.4 μg/kg bw could be derived from in vitro MIC susceptibility testing and bioavailability studies.

\textit{Evaluation}

The Committee considered the toxicological effects of lasalocid sodium to be the most relevant for the purpose of establishing an ADI. A toxicological ADI of 0–5 μg/kg bw was established based on the NOAEL of 0.5 mg/kg bw per day from the developmental toxicity study in rabbits and the multigeneration reproductive toxicity study in rats, with application of an uncertainty factor of 100 for interspecies and intraspecies variability.

\textit{Residue evaluation} [to be prepared by FAO]
Summary and conclusions

Studies relevant to risk assessment

<table>
<thead>
<tr>
<th>Species / study type (route of administration)</th>
<th>Doses (mg/kg bw per day)</th>
<th>Critical end-point</th>
<th>NOAEL (mg/kg bw per day)</th>
<th>LOAEL (mg/kg bw per day)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mouse</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Two-year study of toxicity and carcinogenicity (diet)</td>
<td>0, 1.5, 5.25, 18</td>
<td>No relevant findings</td>
<td>18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>—</td>
</tr>
<tr>
<td><strong>Rat</strong></td>
<td></td>
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<tr>
<td>Thirty-month study of toxicity and carcinogenicity (diet)</td>
<td>Males: 0, 0.5, 1.8, 6.2, Females: 0, 0.6, 2.2, 8.1</td>
<td>Increased incidence of impaired righting and grasping reflexes in females</td>
<td>2.2</td>
<td>8.1</td>
</tr>
<tr>
<td>Multigeneration reproductive toxicity study, including teratogenicity study (diet)</td>
<td>0, 0.5, 1.75, 6</td>
<td><strong>Parental toxicity: Reduced numbers of corpora lutea and implantations</strong></td>
<td>0.5&lt;sup&gt;*&lt;/sup&gt;</td>
<td>1.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Embryo and fetal toxicity: Decreased fetal weights and increased incidence of visceral and skeletal variants</td>
<td>1.75</td>
<td>6</td>
</tr>
<tr>
<td><strong>Rabbit</strong></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Developmental toxicity study (gavage)</td>
<td>0, 0.5, 1, 2</td>
<td>Maternal toxicity: Decreased body weight gain, decreased feed consumption and altered faecal output</td>
<td>—</td>
<td>0.5&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Embryo and fetal toxicity: Decreased litter weights, increased incidence of forelimb flexure and minor skeletal abnormalities/variants</td>
<td>0.5&lt;sup&gt;*&lt;/sup&gt;</td>
<td>1</td>
</tr>
<tr>
<td><strong>Dog</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Two-year toxicity study (dietary)</td>
<td>0, 0.25, 1, 5</td>
<td>Transient intermittent paralysis of limbs and increased serum alkaline phosphatase</td>
<td>1</td>
<td>5</td>
</tr>
</tbody>
</table>

<sup>a</sup> Highest dose tested.
<sup>b</sup> Lowest dose tested.
<sup>c</sup> Maternal toxicity was likely due to the sensitivity of rabbits to antibacterial effects on the microflora of the gastrointestinal tract. It is not considered appropriate to consider the maternal toxicity in relation to derivation of an ADI.

*Pivotal study value (14, 15)*

Uncertainty factor

100 (10 for interspecies variability and 10 for intraspecies variability)

Toxicological effects

A toxicological ADI of 0–5 µg/kg bw could be derived.

Microbiological effects

A microbiological ADI of 0–8.4 µg/kg bw could be derived.
ADI (based on toxicological effects)
0–5 µg/kg bw

Residue definition [prepared by FAO]

MRLs [prepared by FAO]

Estimated dietary exposure [prepared by FAO]
Annex 5: Standard phrases for the report item

Why on agenda

[Compound] has not previously been evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The Committee evaluated [compound] at the present meeting at the request of the [Xth] Session of the Codex Committee on Residues of Veterinary Drugs in Foods (FAO/WHO, 20xx) with a view to establishing an acceptable daily intake (ADI) and recommending maximum residue limits (MRLs) in [animal species] tissues.

or

[Compound] was previously evaluated by JECFA at its [xth, yth and zth] meetings (Annex 1, references x, y and z). It is in the agenda on the request of…. to do (what) …

GLP statement

All critical studies contained statements of compliance with GLP.

or

All critical studies contained statements of compliance with GLP, unless otherwise specified.

or

Some of the critical studies do not comply with GLP, as the data were generated before the implementation of GLP regulations. Overall, however, the Committee considered that the database was adequate for the risk assessment.

Carcinogenicity

[when compound shows no evidence of carcinogenicity]

The Committee concluded that [compound] is not carcinogenic in mice or rats.

or

[when compound shows evidence of carcinogenicity in one test species but not the other one]

The Committee concluded that [compound] is carcinogenic in mice/rats but not rats/mice.

or

[when compound shows evidence of carcinogenicity in one sex of one test species but not in the other sex or the other test species]

The Committee concluded that [compound] is carcinogenic in [female/male] rats but not [male/female] rats or mice.

or

The Committee concluded that [compound] is carcinogenic in [female/male] mice but not [male/female] mice or rats.

Genotoxicity

[when no positive/equivocal results]
[Compound] was tested for genotoxicity in an adequate range of assays, both in vitro and in vivo. No evidence of genotoxicity was found.

The Committee concluded that [compound] is unlikely to be genotoxic.

or

[when positive/equivocal in vitro results]

[Compound] was tested for genotoxicity in an adequate range of assays, both in vitro and in vivo. It gave a positive/equivocal response in the in vitro [names of assays], but it was negative in the in vivo [names of assays]. [There is considerable flexibility here in the description of the positive-equivocal test results]

The Committee concluded that [compound] is unlikely to be genotoxic in vivo.

or

[when positive in vivo and in vitro results]

[Compound] was tested for genotoxicity in an adequate range of assays, both in vitro and in vivo. It gave a positive response in the in vitro [names of assays] and in the in vivo [names of assays]. [There is considerable flexibility here in the description of the positive-equivocal test results]

The Committee concluded that [compound] is genotoxic.

or

[when database is inadequate]

The Committee concluded that the available database on the in vivo and in vitro genotoxicity of [compound] was inadequate to allow a conclusion on genotoxicity.

Carcinogenicity and genotoxicity

[when not carcinogenic and no positive genotoxicity results]

In view of the lack of genotoxicity and the absence of carcinogenicity in mice and rats, the Committee concluded that [compound] is unlikely to pose a carcinogenic risk to humans.

or

[when not carcinogenic and positive in vitro genotoxicity results]

In view of the lack of genotoxicity and the absence of carcinogenicity in mice and rats, the Committee concluded that [compound] is unlikely to pose a carcinogenic risk to humans at doses relevant to residues of veterinary drugs.

or

[when carcinogenic and no positive genotoxicity results]

In view of the lack of genotoxicity, the absence of carcinogenicity in [species] and the fact that only [tumours] were observed and that these were increased only in [sex] [species] at the highest dose tested, the Committee concluded that [compound] is unlikely to pose a carcinogenic risk to humans at doses relevant to residues of veterinary drugs. [There is considerable flexibility in the wording used here.]
As [compound] is unlikely to be genotoxic in vivo and there is a clear threshold for [tumour type] in [sex] [species], the Committee concluded that [compound] is unlikely to pose a carcinogenic risk to humans at doses relevant to residues of veterinary drugs. [There is considerable flexibility in the wording used here.]

or

As [compound] is genotoxic in a variety of in vivo and in vitro tests and there is no clear threshold for [tumour type] in [sex] [species], the Committee concluded that [compound] should be considered a carcinogen acting by a genotoxic mode of action.

Teratogenicity

[when compound shows no evidence of teratogenicity in either test species]
The Committee concluded that [compound] is not teratogenic in rats or rabbits.

or

[when compound shows evidence of teratogenicity in one test species but not the other one]
The Committee concluded that [compound] is teratogenic in rats but not rabbits.

or

The Committee concluded that [compound] is teratogenic in rabbits but not rats.

Neurotoxicity

[when compound shows no evidence of neurotoxicity in the species tested]
The Committee concluded that [compound] is not neurotoxic.

or

[when compound shows evidence of neurotoxicity in the species tested]
The Committee concluded that [compound] is neurotoxic.

Microbiological effects not of concern

[when the Committee determines that microbiological effects are not anticipated for the compound]
Considering the chemical structure and the mode of action of [compound], the Committee did not anticipate any adverse effects of [compound] residues on the human gastrointestinal microbiota.

ARfD unnecessary

[when the Committee determines that an ARfD is unnecessary for the compound]
The use profile of [compound] as a veterinary drug is such that dietary exposure to [compound] from a large portion is unlikely to be markedly greater than that from chronic consumption. The toxicological profile of [compound] is such that it is unlikely to present an
acute hazard. The Committee therefore concluded that it was not necessary to assess the acute risk from exposure to [compound] when used as a veterinary drug.

**Preparation of monograph**

A toxicological monograph was prepared.

*or*

A toxicological monograph was not prepared. *to be used if no data were provided, but compound was briefly discussed at meeting*

*or*

An addendum to the toxicological monograph was prepared. *to be used if a toxicological monograph had been prepared for the compound at a previous meeting*

*or*

An addendum to the toxicological monograph was not prepared. *to be used if no new data were provided for a compound for which a toxicological monograph had been prepared at a previous meeting and which was discussed briefly at the current meeting*